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PATHOLOGY

A Periodical Devoted to General and Experimental Pathology

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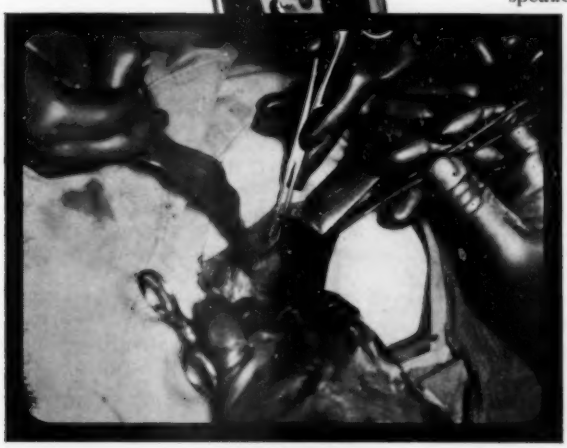

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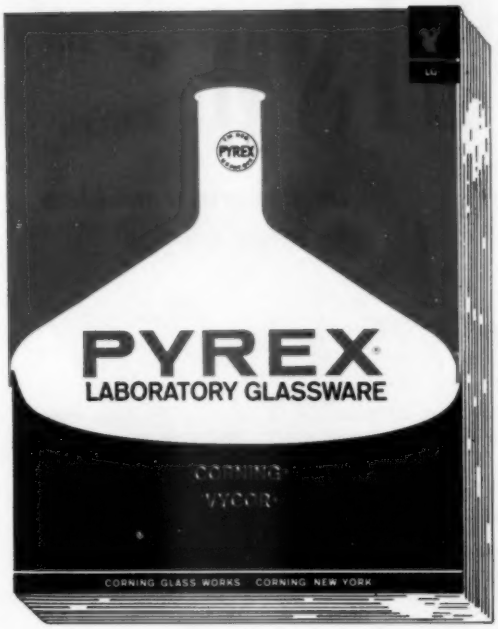

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Cells of Human Heart and Aorta Grown in Tissue Culture

ESTELLE FASOLINO INGENITO, Ph.D.; JOHN M. CRAIG, M.D.; JACQUELINE LABESSE, M.D.; MARTHE GAUTIER, M.D., and DAVID D. RUTSTEIN, M.D., Boston
With the Technical Assistance of Mary Lou Foley and Burton Goldberg

The study of the mechanism of two diseases, rheumatic fever and atherosclerosis, is being furthered by the development of standard cultures of adult human heart and aortic tissues, and reports of the experiments on atherosclerosis are now in press.[1] Incorporating human serum in the medium makes it possible to perform critical experiments by substituting serum obtained from the blood of patients with rheumatic fever and with atherosclerosis. This paper reports the development of standardized cultures in such a medium. In 86 different experiments the method has never failed to produce growth. These cultures have been maintained for as long as 18 months, at which time they were still actively proliferating.

Submitted for publication Sept. 24, 1957.

From the Department of Preventive Medicine, Harvard University Medical School (Dr. Ingenito), and from the Department of Pathology, Children's Medical Center (Dr. Craig).

These studies were made possible by a grant, No. 5578-2, from the American Heart Association and the Marie-Laure Fund established by M. and Mme. Pierre Lepaulle of Paris.

Dr. Robert E. Gross, Surgeon-in-Chief, Children's Medical Center; Dr. Dwight E. Harken, Surgeon and Chief of the Department of Thoracic and Cardiac Surgery, Peter Bent Brigham Hospital, and Dr. John Gordon Scannell, Associate Visiting Surgeon, Massachusetts General Hospital, provided a continuing supply of aortic and human heart fragments obtained at operation.

Methods and Materials

The cultures were planted with fragments of heart tissue excised at operation from 30 patients with congenital atrial septal defect and from 43 patients with rheumatic mitral stenosis, and of aortic tissue from 13 patients with coarctation of the aorta.

The tissue specimen when excised was collected under sterile conditions, kept moist with a few drops of isotonic saline solution, and planted within an hour.* The moist tissue fragment, with a minimum amount of handling, was washed free of adherent blood and fat with a few drops of the fluid nutrient medium (see below) and was then cut into smaller fragments. These tissue fragments were cut into pieces about 1.5 mm. square, with use of two No. 7 Bard Parker straight-cutting-edge blades in scissors-like fashion.

Standardization of the size of the cut explants is facilitated by having a small set of appropriately sized white squares on a black card, which is slipped underneath the Petri dish containing the tissue. All blood clots and fatty material are discarded. Care is taken not to tear or shred the tissue during the cutting, as better growth results when the edges are kept smooth. In the case of aortic tissue, the adventitia is separated off and discarded, leaving the translucent inner lining to be planted. Neither the tissue specimen nor the cut explants should be left soaking in the drops of nutrient medium any longer than necessary. They should be planted immediately.

The cultures are planted in Earle's T-15 and T-60 flasks for long-term and roller tube and

* Two specimens of adult human heart tissue were held at refrigerator temperature for 24 hours, and good growth was obtained when the tissue was then later planted in the usual manner.

Maximow slide preparations for short-term experiments. The tissue culture is prepared in Earle's T-15 flasks as follows:

1. Two drops of chicken plasma are spread evenly and thinly over the entire floor of the flask. Careful spreading is especially important if photomicrography of the tissue culture is contemplated.

2. The flask is placed at a slight angle for five minutes to allow any excess plasma to drain down into the bottom, from where it is removed.

3. Four explants are transferred from the Petri dish and placed in the center portion of the flask in the form of a square—about 1.5 cm. apart, 0.7 cm. from the side edges of the flask, 2 cm. from the bottom point of the flask, and 1 cm. below the constriction at the bottom of the neck of the flask.

4. Two drops of a mixture of 40% human serum, 20% chick embryo extract 50, and 40% Hanks' balanced salt solution is added, and the flask tilted to distribute the clotting medium evenly over the explants and to mix it thoroughly with the plasma on the floor of the flask, thereby inducing the formation of a thin clot.

5. After the clot formation, the flask is placed at a slight angle to allow drainage into the bottom, and the excess is then removed and discarded.

6. When the clot is firm, the flask is stoppered with a nontoxic stopper and placed with the explants "on the floor" overnight at 37.5 C.

7. After overnight incubation, the preparations are examined. If no explants have become loosened, the accumulated drainage is removed and the cultures are immediately fed with the fluid nutrient medium. If explants have become loosened, these are patched with chicken plasma and nutrient medium to form a fresh clot. They are fed after reincubation at 37.5 C for one hour.

The nutrient medium consists of a mixture of 40% adult human blood serum (RH-negative, Type O, or RH-positive, Type AB), 20% chick embryo extract 50, and 40% Hanks' balanced salt solution. The medium also contains phenol red indicator (0.002%) and 100 units of penicillin G and 50μg of streptomycin sulfate per milliliter of medium.

For each T-15 flask, 20 drops of the freshly prepared nutrient medium are added gently upon the explants and the clot. The flask is carefully tilted to break the surface tension of the medium over the clot and the adhering tissue fragments and to permit all of the explants to be bathed in the medium. The flask is then restoppered with a fresh sterile stopper, and the culture preparation is reincubated at 37.5 C, with the explants "on the floor" covered with fluid medium.

The T-60 flask preparations are prepared in exactly the same manner. Five drops of chicken plasma and five drops of the clotting medium are used, and as many as 40 or 50 explants can be placed in one flask. One hundred drops of fluid nutrient medium is used for each T-60 flask. The T-60 flask cultures have been especially useful for the maintenance of stock cultures and when large numbers of tissue explants are required for a given experiment.

Early in the period of growth, the fluid medium is usually changed three times weekly. The old medium is removed, the cultures are washed with 2 to 3 drops of freshly prepared fluid nutrient medium, and the appropriate volume of fresh medium is added. In the interim between feeding periods, if the supernatant nutrient fluid becomes acid due to the metabolism of the tissue, the pH is adjusted by the addition of 1 or 2 drops of 1.4 isotonic sodium bicarbonate at pH 7.6. One or two drops of a 1% glucose solution is also added at this time.

Results

After the intial planting, the initial growth in the plasma surrounding the embedded tissue is usually noted within two to eight days for the human adult heart and within three to four weeks for the human aortic tissue fragments. The rate of growth of the human aortic tissue fragment has been variable, some cultures growing out within several weeks, with others failing to grow until transfer was made one month after the original planting.

After the first evidence of growth, the area of growth increases for two to six weeks, the cells migrating out to the edges of the floor of the flask. At this stage, individual or small aggregates of cells will detach themselves from the uniform sheet and migrate into other areas of the flask and form new growth centers. These islets may be left in the original culture vessel. When the original explants are transferred to subculture flasks, they continue to proliferate both on the old clot and on the surface of the glass.

The outgrowth from the adult human heart tissue consists of elongated bipolar cells which are arranged in irregular networks or in radiating strands (Fig. 1).

Fig. 1.—Some typical heart muscle cells (eighth transfer, routine technique). Hematoxylin and eosin.

These long spindle-shaped cells grow in a monocellular layer and measure up to about 200μ in greatest diameter. These mononuclear cells show considerable variation in size of both cytoplasm and nucleus, the latter containing one or more prominent nucleoli.

In contrast, in the aortic tissue cell cultures the cells grow in much more sheet-like fashion and are one layer in thickness (Fig. 2). In the outgrowth, the majority of the cells tend to have round borders with plump cytoplasmic masses. At the periphery of the area of the outgrowth, the cells become more elongated with thin cytoplasmic processes. Mitotic figures are frequent, but no multinucleated cells are present.

One of us (J. M. C.) has studied the cells in the outgrowth in an attempt to discover their exact identity. The cells are clearly primitive ones, but it is impossible to further identify them. The cells in the outgrowth in the heart tissue cultures are usually more spindle-shaped than the more cuboidal aortic cells; however, since it is possible by subculture from the same tissue culture of either heart or aorta to obtain cells of different morphologic appearance in different subcultures, it is impossible to state that the cells in the outgrowth of the two tissues differ from each other. The much more rapid growth of cells in the heart tissue cultures with use of the same culture medium suggests that these cells differ from the aortic ones, but without further evidence no definite statement can be made.

When the growth covers the entire floor of the flask, subculture becomes necessary. Subculture may also be required if the clot becomes opaque, thus preventing photomicrography. The standard procedures described in the literature [2] for the transferral of cell cultures have been successfully used here.

At the time of transfer, cells have been left on the glass and on the old clot in the flask. These remaining cells will continue to proliferate for a few weeks and finally reach a point where they no longer grow. However, they remain alive in this dormant state for as long as six months if

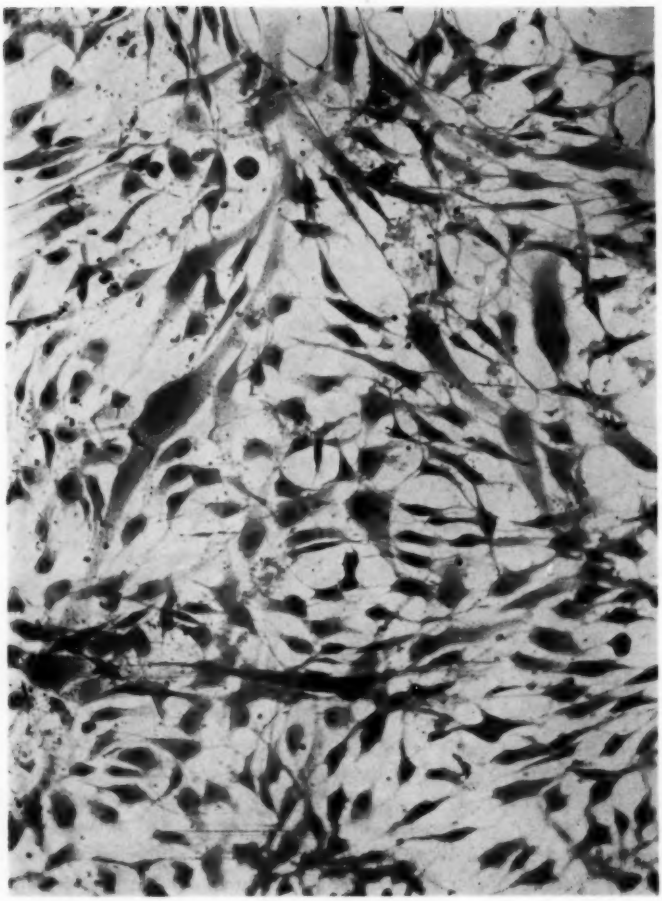

Fig. 2.—Some typical aorta cells (eighth transfer, routine technique). Hematoxylin and eosin; reduced about 15% from mag. × 210.

the medium is changed once a week and the clot is patched with chicken plasma and nutrient medium as required.

This technique has been modified for use in particular experiments where photomicrography of the cells is crucial or where the clot interferes with chemical analyses of the fluid medium. In this modified technique, when transfers are made as described above, the cut fragments are placed directly onto the glass floor of the T-15 flasks. The flask is then turned upside down, with care being taken not to dislodge the explants. Twenty drops of a mixture of 50% adult human serum and 50% Hanks' balanced salt solution is placed in a flask, with care being taken that the medium does not touch the explants. A sterile stopper is inserted, and the flask is incubated overnight at 37.5 C, with the explants on the upper surface of the flask. After overnight incubation, the fluid medium is removed and the flask is held with the explants "on the ceiling." The culture is then fed with 20 drops of freshly prepared medium containing either 10% human adult serum with 90% Eagle's synthetic medium containing glutamine or 50% human adult serum and 50% balanced salt solution.

The modified procedure has been successful, even with fresh fragments of adult human heart and aortic tissue prepared for planting in the usual manner. In this modified technique, the medium must be renewed twice weekly. Subsequent transplantations

can be made with use of the standard procedure.

Summary

Cultures of cells from human heart and aorta have been successfully grown and maintained in a medium containing human blood serum.

The cultures of human heart were grown from specimens of tissue obtained at operation from 30 patients with congenital atrial septal defect and from 43 patients with rheumatic mitral stenosis. These cultures contain cells of a characteristic morphological appearance.

Cultures of cells have been successfully grown and maintained from specimen of the lining of the adult human aorta obtained at operation from 13 patients with coarctation of the aorta. These cells have morphological characteristics which differ from those grown from specimens of adult human heart tissue.

Two techniques are described, one with and the other without a plasma clot. With use of the original method with a plasma clot, the parent and daughter cultures of human heart and aortic tissues have been grown and maintained for as long as 18 months, at which time the cells were still actively proliferating.

These standardized cultures are useful for in vitro experiments on rheumatic fever and atherosclerosis.

Department of Preventive Medicine, Harvard University Medical School (15) (Dr. Rutstein).

Addendum

Since we submitted this article for publication, it has been possible to establish a cell line of aortic cells which thus far has gone three generations. Explants are removed from a T-60 flask, and the remaining clot containing aortic cells is covered for 15 minutes with a solution of 0.25% trypsin (Difco) in Hanks' balanced salt solution at room temperature. The resulting suspension is centrifuged at 600 rpm for one minute; the supernatant is decanted, and the sediment is resuspended in 5 ml. of normal medium. A satisfactory culture is obtained on glass by placing 1 ml. of the cell suspension in a T-15 flask. Cultures have also been obtained on clot by covering the floor of the flask with 1 drop of chicken plasma and 1 drop of the cell suspension in the normal medium. After a clot is formed, 1 ml. of the cell suspension is then introduced into the flask. Adequate growth appears within two to four days at 37 C.

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Effects of Sulfated Alginic Acid (SAA) on Preestablished Rabbit Atherosclerosis

II. Study in the Absence of Concomitant Cholesterol Feeding

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Introduction

It has been demonstrated that sulfated alginic acid (SAA) treatment, started in cholesterol-fed rabbits some time after the development of measurable atherosclerosis and carried on in the presence of a continued high cholesterol intake, will arrest the progress of the disease.[1]

Under the same conditions, while abolishing the visible lipemia and reducing the hypercholesterolemia, SAA increased the uptake of fat by reticuloendothelial cells and it caused the appearance of lipophages in renal glomeruli. Furthermore, this compound induced in several aortae the appearance of plaques of low histological lipid content, some of them umbilicated and overlying calcified medial patches.

The purpose of the present study was to find out whether SAA will influence the slow spontaneous regression of lesions that follows discontinuation of cholesterol feeding,[2] with special attention to histological changes.

Materials and Methods

Fifty New Zealand White rabbits were given a 1% cholesterol diet for two months. At the end of this period, the 33 animals that achieved comparable serum turbidity and cholesterol levels were divided into three groups of 11 rabbits each and matched with regard to lipemia, sex, and litter, whereas the remaining 17 animals were discarded.

Group I was killed immediately, to serve as a witness of the amount of atherosclerosis that had developed after two months' cholesterol feeding.

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In Groups II and III cholesterol feeding was discontinued and the rabbits were given a normal diet for another two months before they were killed, but Group III also received daily injections of SAA (5 mg. per kilogram subcutaneously) throughout the cholesterol-free feeding period. Up to the time the animals in Group I were killed, all animals were offered 80 gm. of the high-cholesterol diet daily, with registration of the voluntary food intake. Groups II and III were pair-fed.

In every animal, the wet weight, the total atheroma area, and the total cholesterol content of the aorta from the valves to the diaphragm were determined as previously,[1] while the abdominal aorta (from diaphragm to iliac bifurcation) was sampled separately for total atheroma area and histological study only. In addition, a careful examination for incidence of umbilicated atheromata was made in each instance.

Three formalin-fixed samples obtained from the center of the highest plaque in the ascending, thoracic, and abdominal aorta, respectively, were examined microscopically in every rabbit.

In each of these three lesions in turn, the following six features were recorded: (1) the maximum thickness, in microns, from the first intact elastic membrane to the highest point, or "crest," of the plaque; (2) the maximum width, in microns, of the lipid-free surface zone, wherever present; (3) the incidence of sudanophilic lipid migration across the internal elastic membrane into the media; (4) the incidence of calcification or deposition of basophilic material in the underlying media; (5) the density of the sudanophilic content, and (6) the predominantly extracellular or intracellular location of the sudanophilic material.

Of the above histological end-points, the first four represented objective records, whereas the latter two were semiquantitative gradings on the basis of arbitrary criteria.

With regard to "sudanophilic density," Grades 1, 2, 3, or 4 indicated that sudanophilic material was occupying less than half, approximately half, more than half, or the entire area of the plaque, respectively.

TABLE 1.—*Serum Turbidity and Cholesterol* *

Group	High-Cholesterol Diet Period			Cholesterol-Free Diet Period		
	Serum Turbidity		Serum Cholesterol	Serum Turbidity		
	Day 0	Day 64	Day 64	Day 76	Day 83	Day 98
I	6±1.7	31±9.3	791±136			
II	6±1.7	31±5.9	826±122	12±1.9	10±1.8	10±1.4
III	6±1.7	31±5.0	889±143	7±1.8	5±1.1	4±1.0

* Turbidity is expressed in Klett units; cholesterol, in mg./100 ml. On Day 0 all animals started the cholesterol diet. On Day 70 Group I was killed, while Groups II and III switched to a normal diet.

With regard to "extra- or intracellular location of sudanophilic material," every plaque was classified under one of the following two headings: (1) extracellular sudanophilic component equal to or greater than the intracellular component ("E>I"), and (2) extracellular sudanophilic component smaller than the intracellular one ("E<I").

The standard microscopic examinations were made in 10μ thick frozen sections, prepared from the center of the formalin-fixed samples. Two adjacent frozen sections were secured from each plaque, one stained with Sudan red and hematoxylin, the other with Von Kossa's silver nitrate procedure for calcium. Where indicated, a few additional paraffin sections were made and stained with Hale's colloidal-iron technique or toluidine blue.

Periodic serum turbidity determinations throughout the experiment, a single serum cholesterol determination at the end of the cholesterol-feeding period, and the assessment of coronary atherosclerosis were all as previously described.[1]

Standard Sudan red-and-hematoxylin-stained frozen sections and hematoxylin-and-eosin-stained paraffin sections were prepared from every kidney (entire organ) and every spleen (midportion).

Body weights were taken at weekly intervals throughout the duration of the experiment.

Results

1. *Serum Turbidity and Cholesterol* (Table 1).—All three groups achieved the same mean serum turbidity and serum cholesterol concentration at the end of two months' cholesterol feeding. This was, of course, the result of randomizing the distribution of rabbits in the three groups and discarding those with extremely low or extremely high lipemic responses at the end of the cholesterol-feeding period. Discontinuation of cholesterol feeding resulted in a rapid decline of the serum turbidity in both Group II and Group III. SAA, however, accelerated significantly the return toward normal of the serum turbidity value in Group III.

2. *Aortic Wet Weight, Cholesterol Content, Total Atheroma Area, and Umbilicated Lesions* (Table 2).—There was no significant difference between the wet weight, the cholesterol content, or the total atheroma area of any two groups. There was similarly no group predilection in the incidence of umbilicated lesions, which was as follows: Group I, 3 out of 11; Group II, 3 out of 11; Group III, 4 out of 11. Among the animals displaying umbilicated plaques, only those with kidney damage showed a marked degree of this change, the others exhibiting a negligible involvement (Item 8, below).

3. *Histological Features of Aortic Lesions* (Table 3).—(*a*) Maximal Thickness: There were no significant differences in the maximal thicknesses of the plaques in any aortic segment of the three groups.

TABLE 2.—*Aorta and Coronaries*

Group	Aorta				Coronaries		
	Arch and Thoracic			Abdominal Area Occupied by Plaques, %			
	Wet Weight, Mg.	Area Occupied by Plaques, %	Cholesterol Content, Mg./Aorta		Coronaries Encountered/ Section, Total No.	Atheromatous Coronaries, %	Completely Occluded Coronaries, %
I	814±127	33.8±11	8.5±3.6	17.0±4	29±1.0	17±3.8	7±2.0
II	812±123	40.0±10	12.6±4.0	23.2±4	33±2.9	21±4.4	10±2.1
III	831±104	41.7±7	11.9±5.0	18.4±5	32±2.4	17±3.5	7±4.7

TABLE 3.—*Histological Characteristics of Aortic Lesions*

Group	Maximum Thickness, μ	Sudanophilic Density, Grade	Ratio of Extra to Intracellular Lipid, E/I		Maximum Width of Superficial Lipid-Free Zone, μ	Lipid Migration into Media, Incidence	Subplaque Medial Calcification, Incidence	Subplaque Medial Basophilia, Incidence
			E≥I	E<I				
				Ascending Aorta				
I	213±47	2.3±0.3	5/11	6/11	16.7±9	9/11	2/11	4/11
II	300±32	2.7±0.2	4/11	7/11	24.0±9	8/11	1/11	2/11
III	290±49	1.9±0.3	2/11	9/11	56.2±26	8/11	2/11	4/11
				Thoracic Aorta				
I	156±38	2.6±0.1	6/11	5/11	2.5±2	7/11	1/11	3/11
II	231±27	2.5±0.1	8/11	3/11	4.0±3	7/11	1/11	2/11
III	221±36	2.4±0.2	5/11	6/11	13.3±10	5/11	1/11	2/11
				Abdominal Aorta				
I	170±20	2.2±0.2	9/11	2/11	31±10	4/11	0/11	4/11
II	250±31	2.0±0.2	7/11	4/11	14±6	7/11	2/11	3/11
III	210±38	1.5±0.2	4/11	7/11	49±14	9/11	0/11	3/11

(*b*) Sudanophilic Density. There were no significant differences in the lesion densities of the three groups in the ascending or thoracic aortic segments. In the abdominal aortic segment, however, the lesions of Group III exhibited a trend toward a lower density than those of Group I ($P=0.05-0.02$), whereas those of Group II were not significantly different from either Group I or Group III.

(*c*) Extracellular/Intracellular Lipid Distribution Pattern: By the χ^2-test there was no significant difference between the lipid distribution patterns of the lesions of Groups I and II in any aortic segment or Groups I and III in the ascending and thoracic segments. In the abdominal segment, however, the lesions of Group I presented a predominantly extracellular pattern, whereas those of Group III were predominantly of the intracellular type, the difference being of borderline significance ($P=0.05-0.02$). Examples of extreme variations in sudanophilic patterns of plaques are shown in Figures 1 and 2.

(*d*) Maximal Width of Superficial Lipid-Free Zone and Incidence of Lipid Migration: Although the highest values generally occurred in Group III, there was no significant difference between any two groups with regard to these two criteria.

(*e*) Subplaque Medial Calcification and Deposition of Basophilic Material: There was a low incidence of medial calcification underneath plaques, without predilection for any particular group. Wherever it was seen, such focal calcification was accompanied by the deposition of a basophilic material which was Hale-positive and metachromatic to toluidine blue. As can be seen in Table 3,

Fig. 1.—Example of an atheroma with "Grade 4" sudanophilic density and no lipid-free surface zone. The sudanophilic material is partly intracellular (formed dark masses) and partly extracellular (pools of amorphous gray between the dark lipophages). From the abdominal aorta of an animal in Group I. Frozen section, Sudan red and hematoxylin; × 140.

Fig. 2.—Example of an atheroma with "Grade 1" sudanophilic density and predominantly intracellular lipid distribution pattern. The upper third of the plaque consists of lipid-free tissue. The dark spots in the upper third represent blue-staining nuclei of lipid-free cells, whereas the dark spots in the lower two thirds represent red-staining lipid-filled cells. From the abdominal aorta of an animal in Group III. Frozen section, Sudan red and hematoxylin; × 140.

subplaque deposits of this basophilic material were sometimes encountered alone, without calcification. Almost invariably, the elastic lamellae within such calcified or basophilic areas were distorted and in various stages of destruction, while the cells between the lamellae were in various phases of degeneration and cytolysis. Typical findings are illustrated in Figures 4 and 5.

All animals exhibiting medial calcification in one or other aortic segment were found to have kidney damage (Item 8, below).

(*f*) Intraplaque Calcification: Calcification within the intimal plaques, as opposed to calcification of the media underneath them, was seen only in two animals of Group II and one animal of Group III. All three animals were among those that exhibited kidney damage (Item 8, below). Figure 3 shows such calcium deposits in the center of an unusually thick lesion.

4. *Coronaries* (Table 2).—There was no significant difference between any two groups with regard to incidence or severity of coronary atherosclerosis.

5. *Spleen* (Table 4).—There were no significant differences in the splenic weights of the three groups. Some animals of Group I developed a mild degree of splenic foam-cell infiltration, but none was seen in the spleens of the other two groups.

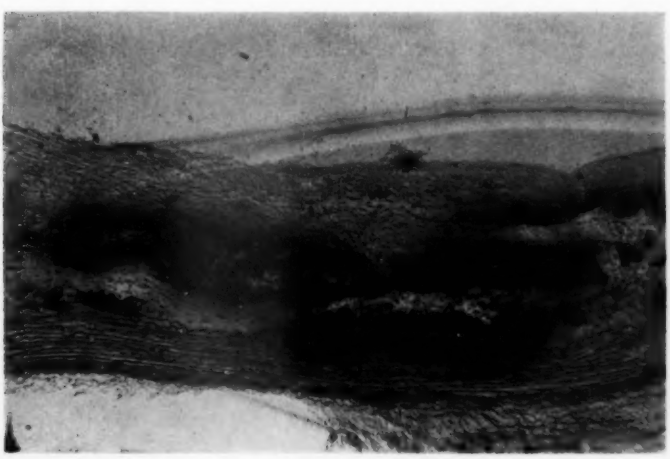

Fig. 3.—Intraplaque calcification of an extremely thick atheroma from the abdominal aorta of an animal in Group II with kidney damage. Frozen section, Von Kossa stain; × 49.

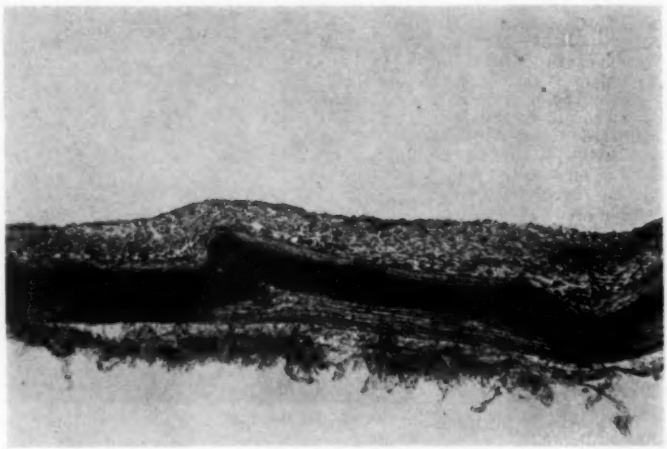

Fig. 4.—Confluent atheromatosis and media necrosis in the abdominal aorta of an animal from Group I with kidney damage. The atheroma is of the low-density, predominantly intracellular type. The dark basophilic band within the media represents a rigid plate containing both Hale-positive material and calcium; it corresponds to the area of destruction of elastic lamellae and muscle. Note the crack at the junction of the left and middle third of this plate, with partial overriding of the "fragments"; it presumably represents an artifact caused by postmortem contraction of the aortic wall. Frozen section, Sudan red and hematoxylin; × 49.

6. *Kidney* (Table 4).—No glomerular foam cells developed in any kidney of this experiment, and there were no significant differences between the mean kidney weights of the three groups.

The renal parenchyma was histologically normal in all except seven rabbits (two each in Groups I and II and three in Group III). In these exceptional animals assorted renal lesions appeared, ranging from tubular atrophy, dilatation, and calcification to casts, scarring, and glomerular obliteration. A typical example of a kidney presenting most of these changes is shown in Figure 7. It should be noted that were it not for the routine histological examination of every kidney the presence of renal pathology might have remained undetected in some animals.

Unfortunately, the etiology of the observed renal changes could not be determined in this experiment. Of the seven animals with renal damage, three had exhibited frequent bouts of diarrhea throughout the duration of the experiment; one had developed a pyogenic abscess in the neck; another, a pneumonic consolidation in the right lung, and no gross pathology was vis-

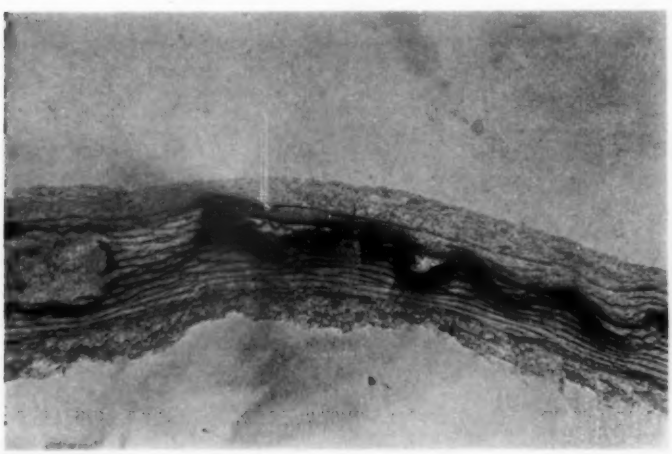

Fig. 5.—Same material as in Figure 4, adjacent frozen section stained with Von Kossa's stain to show the calcium component of the basophilic band in the media and the elastic fiber distortion and destruction in the same area. Note also the calcium impregnation of individual elastic lamellae; × 49.

TABLE 4.—*Spleen, Kidney, and Total Body Weights*

Group	Spleen Wt., Mg./Kg. Body Wt.	Spleen Foam-Cell Infiltration, Incidence	Kidney Wt., Gm./Kg. Body Wt.	Kidney Foam-Cell Infiltration, Incidence	Initial Body Wt., Kg.	Body Wt., at Day 70, Kg.	Terminal Body Wt., Kg.
I	428±41	4/11	3.58±0.21	0/11	2.69±0.07	3.06±0.09	3.06±0.09
II	382±63	0/11	3.42±0.22	0/11	2.58±0.11	3.08±0.08	3.17±0.10
III	368±38	0/11	3.37±0.23	0/11	2.55±0.11	3.01±0.10	3.15±0.13

ible in the last two animals. Thus, in some of the animals that developed it, the kidney damage might have been of infectious (toxemic) origin, although an alternative pathogenesis with secondarily increased susceptibility to infection could not be ruled out.

7. *Growth and Clinical Condition* (Table 4).—With the exception of the few rabbits that developed renal damage, the animals of all three groups consumed the entire amount of food offered them daily, and they gained weight (at the same average rate) during the cholesterol-feeding period. SAA treatment did not affect the food intake or the growth rate of the animals to which it was given. The great majority of all animals remained free of intercurrent infections and exhibited grossly normal viscera at autopsy.

8. *The Exceptional Rabbits with Kidney Damage* (Tables 5 and 6).—These animals exhibited such striking common features—regardless of which group they belonged to—that they merit special consideration.

All seven had voluntarily restricted their food intake for several weeks during the cholesterol-feeding period and thereafter, with resulting loss of body weight. All seven aortae were gritty to palpation and expanded, with gaping innominate, coronary, and other orifices. Their entire intimal surface was occupied by large confluent atheromata, many of them umbilicated. Upon microscopic examination, their media proved extensively calcified and (or) permeated by a basophilic material which was Hale-positive and metachromatic to toluidine blue. Without exception, these aortae were unusually heavy and they had an extraordinarily high cholesterol content. Some of their typical histological findings are illustrated in Figures 4 and 5, while Table 5 summarizes their principal quantitative characteristics.

As can be seen in Table 6, despite a marked change of profile and a reduction of mean and standard error values, the exclusion of the aortic characteristics of these exceptional rabbits did not significantly affect the intergroup relationships that prevailed when they were included (Table 2). This was due to the fortuitously balanced

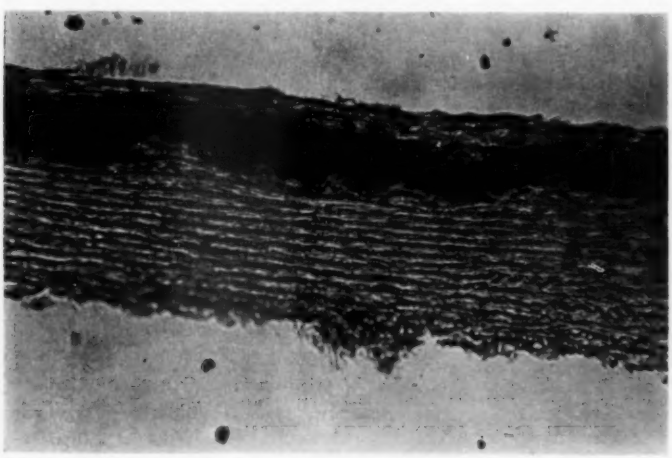

Fig. 6.—Confluent media necrosis without atheromatosis or proliferation of the overlying intima, from a stock rabbit that developed kidney damage while on a normal diet. Calcium, Hale-positive material, and destruction of elastic lamellae have been demonstrated in adjacent sections. Frozen section, Sudan red and hematoxylin; × 140.

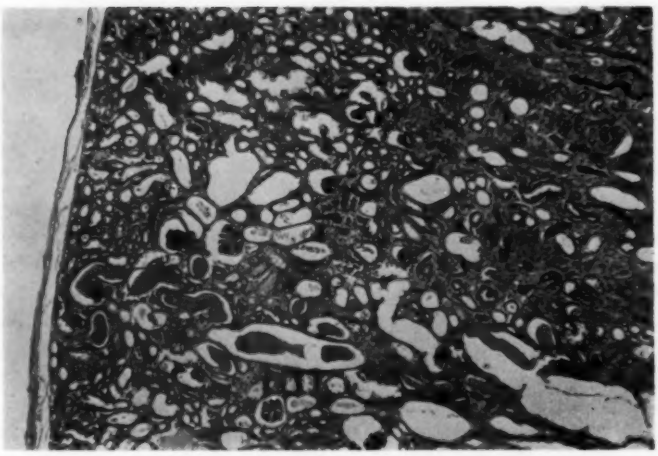

Fig. 7.—Typical kidney damage as seen in the seven experimental and two stock rabbits that developed it. Note the variety of pathological changes, which include tubular dilatation, flattening of tubular epithelium, hyaline casts, tubular and glomerular shrinkage, and scarring. Paraffin section, hematoxylin and eosin; ×49.

TABLE 5.—*Some Aortic Characteristics of the Seven Animals with Kidney Damage* *

Aortic Wet Weight, Mg.	Aortic Area Occupied by Plaques, %	Aortic Cholesterol, Mg./Aorta	Subplaque Medial Calcification, Over-All Incidence	Subplaque Medial Basophilia, Over-All Incidence	Umbilicated Lesions, Over-All Incidence
1570±90	100±0	36±2.9	4/7	7/7	7/7

* The first 3 values refer to aortic arch + thoracic aorta.

incidence of renal damage among the three groups of this experiment. It should be noted that similarly unaffected by exclusion of the exceptional animals were the relationships between the histological parameters of the aortic lesions of the three groups.

It is of considerable interest that shortly after the termination of this experiment a similar syndrome of diarrhea, voluntary food intake restriction, and body weight loss appeared in two stock rabbits that had always been on a normal diet. Upon autopsy, they too exhibited histological kidney damage and extensive calcification (plus deposition of basophilic material) in the aortic media, without, however, any trace of intimal atheromatosis. A typical section through these aortae is shown in Figure 6.

TABLE 6.—*Some Aortic Characteristics Computed After Exclusion of the Values of the Animals with Kidney Damage* *

Group	Aortic Wet Weight, Mg.	Aortic Area Occupied by Plaques, %	Aortic Cholesterol, Mg./Aorta
I	630±40	20.5±8	4.2±1.4
II	671±89	29.0±4	7.0±1.9
III	628±50	20.0±6	4.5±1.4

* All values refer to aortic arch + thoracic aorta.

Comment

The data of this investigation showed that the mere withdrawal of dietary cholesterol for two months failed to produce any significant regression of preestablished plaques in rabbits. Total area, thickness, histological pattern, and cholesterol content of the aortic lesions all remained unchanged after two months of a normal diet. These findings are in line with the earliest histological observations of Anitschkow,[2] who found that it took up to three years of normal dietary regimen before definite sudanophilic depletion and fibrosis of plaques in rabbits occurred. They are also in agreement with more recent work in which, with use of semiquantitative atheroma-area gradings and aortic cholesterol determinations as criteria, no regression was observed after six months [3] or four months [4] of dietary cholesterol withdrawal.

The present data similarly demonstrated that the combination of SAA treatment with cholesterol withdrawal for two months did not cause any regression of lesions in the arch or the thoracic segment of the aorta,

as judged by the multiple criteria applied. SAA treatment did, however, induce certain histological changes in the atheromata of the abdominal aortic segment. These changes consisted of a trend toward a reduction of their sudanophilic material and its increased incorporation in the cytoplasm of scavenger cells—without any diminution in plaque thickness or total area. Since no cholesterol or other lipid determinations were made in the abdominal aortic segment in this study, it is not known whether any significant changes of chemical lipid content paralleled the altered histological pattern of the abdominal lesions. Still, if one considers that abdominal aortic plaques are younger than upper aortic lesions (in rabbits), the above observations raise the possibility that a considerably longer SAA treatment of early plaques might lead to measurable anatomical or chemical regression. An experiment designed to test this possibility is now in progress.

Furthermore, incidental findings of this investigation shed more light on the nature of umbilicated plaques overlying calcified medial patches which were observed sporadically in approximately half the SAA-treated animals of a previous study [1] and which had been tentatively regarded as regressing atheromata. Since these peculiar lesions were prominent only in those rabbits of the present experiment that accidentally developed renal damage (regardless of whether they were treated with SAA or not) and since renal damage in the form of foam-cell-obstructed glomeruli was present in the SAA-treated animals of the previous study,[1] it now seems certain that plaques of this type (*a*) are not caused specifically by sulfated polysaccharides and (*b*) do not represent regressing atheromata. Indeed, in the light of accumulating other evidence it appears likely that the primary event in the genesis of such plaques consists of focal media necrosis-calcification (induced by kidney damage), with a secondary atheromatous response of the intima directly overlying the injured medial patch, the action of the blood pressure accounting for their central depression. In support of this theory stands the known fact that variously induced renal injury will produce aortic media necrosis and calcification in mammals,[5,6] as well as the demonstration that a high-fat diet combined with renal damage (no matter how caused) will produce necrosis, elastic-fiber destruction, and accumulation of Hale-positive material in the subendocardial connective tissue of the dog.[7]

The fact that umbilicated plaques overlying calcified medial patches were also frequent in a heparin-treated group of the previous study,[1] in which no kidney damage could be demonstrated histologically, is not incompatible with the above theory. Since, in addition to renal damage, a host of widely different drugs and stresses have been shown to cause media necrosis and calcification in the rabbit [2,5] and since toxic amounts of heparin had been given during the initial interval of that study, its action on the media was probably nonspecific. At any rate, experimental attempts to reproduce typical umbilicated subcalcified plaques by inducing media necrosis (renogenic or other) in rabbits before and after the development of lipemia are now under way. It is obvious that the definitive elucidation of this phenomenon would help to eliminate a source of error in the interpretation of regression experiments.

Closely related to the above is the observed association between the incidence of kidney damage and media necrosis, on the one hand, and an extreme degree of atherosclerosis, on the other, in animals that had been exposed to cholesterol feeding. The exceedingly high aortic cholesterol content of such animals (regardless of the sudanophilic density of their plaques) was probably due to their extraordinarily high total atheroma area (and presumably volume). Pending further experimental analysis, any explanation for this association can only be speculative. Nevertheless, since no atheromata were seen over the necrotic calcified medial patches of a few animals that

developed renal damage while given a normal diet, it would appear that renogenic media injury greatly potentiates the atherogenic response of the overlying intima to lipemia. Such a hypothesis would be in line with the findings of Wissler et al.[8] that coronary atherogenesis in the rat was greatly increased in the presence of renal damage and with the early report of Anitschkow[2] that epinephrine- or thyroxine-induced medial necrosis increases both permeability and atherogenesis of the neighbouring intima in cholesterol-fed rabbits. Certainly this hypothesis, which is based on accidental side-findings, calls for planned experimental confirmation. Yet even without further analysis, the observations of the present study demonstrate that routine histological examination of the kidney should be included among the end-points of all rabbit atherosclerosis experiments.

Finally, the fact that SAA failed to provoke a splenic and glomerular foam-cell reaction in the present experiment (in which it was given in the absence of simultaneous cholesterol feeding) is in complete harmony with findings made with another sulfated polysaccharide of similar structure, namely, Manuronate (Wyeth).[9,10] As would be expected if the lipid in the foam cells represented part of the excess lipid cleared out of the blood stream, the foam-cell infiltration of various organs elicited by the administration of these compounds to heavily lipemic rabbits proved to be a function, among other things, of the level of fat intake during polysaccharide treatment, appearing only at high cholesterol-intake levels.[10]

Summary

The effect of two months' dietary cholesterol withdrawal on preestablished rabbit atherosclerosis was compared with the action of sulfated alginic acid (SAA) combined with dietary cholesterol withdrawal for the same period of time.

Dietary cholesterol withdrawal alone did not produce any regression of atherosclerosis as judged by total aortic atheroma area, aortic cholesterol content, maximal lesion thickness, or histological pattern of plaques in all aortic segments.

By the same criteria, SAA combined with cholesterol withdrawal failed to elicit significant regression in the lesions of the arch and the thoracic segment, but it tended to change the histological pattern of the plaques of the abdominal aorta without diminishing their thickness or total area.

Under the conditions of the present experiment, SAA did not cause any foam-cell reaction in the spleen or the renal glomeruli, and it did not interfere with normal growth.

Incidental findings indicated that umbilicated plaques overlying patches of calcified media are neither regressing atheromata nor specifically due to SAA action.

The association of accidental renal damage with an unusual atherogenic response of the rabbit aorta has been described.

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Morphologic Effects of Serotonin (5-Hydroxytryptamine)

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Serotonin has been implicated in a variety of physiologic and disease states, and it has been proposed as a therapeutic agent in several conditions. Although many studies have been made of its pharmacologic actions,[1] little is known of its chronic toxicity and its morphologic effects. Such information is important in evaluating any therapeutic agent, and it is of special interest in the case of serotonin because of its possible relationship to the carcinoid syndrome. The carcinoid syndrome, consisting of metastasizing carcinoid tumor of the gastrointestinal tract associated with flushing attacks, cyanosis, dyspnea, diarrhea, often with peptic ulcer,[2] and often with acquired valvular lesions of the heart, has been related to serotonin chiefly through the discovery that carcinoid tumors secrete serotonin[3] and through the observation that patients with the syndrome usually excrete in their urine abnormally large quantities of 5-hydroxyindolacetic acid, a degradation product of serotonin. Animal studies have shown that serotonin is capable of reproducing the clinical features of the syndrome, but its role in causing morphologic alterations has to date been largely speculative. Previous attempts to study the morphologic effects of chronically administered parenteral serotonin have been terminated prematurely because administration to animals in large doses has caused renal cortical necrosis and death.[4,5] The present experiment was undertaken in an attempt to study the role of serotonin in causing morphologic lesions in rats, with particular emphasis on the reproduction of cardiac valvular changes analogous to those of the carcinoid syndrome in humans. The period of study was one year; small doses of serotonin were given initially and were gradually increased as animals proved able to withstand them. Rats were selected as test animals because they had previously been shown to be very susceptible to the drug and because the most effective route of administration in this species has been found to be by subcutaneous injection[1]; this allows for prolonged daily administration without the technical problems and the risk of death or complications from infection that follow the intravenous, intra-arterial, and intraperitoneal procedures. In a number of animals receiving serotonin in this study, fatty liver leading to cirrhosis was produced by a choline-deficient, high-calorie, high-fat diet because in previous observations of humans with metastatic carcinoid tumor[2] it was noted that fatty liver was present in the patients with valvular lesions and because among patients with the carcinoid syndrome all adequately

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documented cases with valvular lesions have also had carcinoid liver metastases, suggesting that liver damage may be of importance in the pathogenesis of the cardiac changes. In addition, serotonin blood levels have been found to be altered in patients with cirrhosis.[1]

Materials and Methods

I. Forty-six 21-day-old female Albino rats of the Sprague-Dawley strain were used.* These were separated by littermates into four groups.

A. Group I (11 rats) received an ordinary diet of grain pellets, bread, cheese, and water ad libitum and in addition were given twice daily subcutaneous injections of 5-hydroxytryptamine (serotonin creatinine sulfate) † for periods of up to 353 days; the amounts of injected serotonin were increased in all test animals as follows. For 3 days 2 mg. was given twice daily (4 mg. per day); for the next 12 days 4 mg. was alternated with 2 mg. doses (6 mg. per day); for the next 8 days 6 mg. doses were alternated with 4 mg. doses (10 mg. per day); for the next 40 days 6 mg. doses were given twice daily (12 mg. per day); thereafter, for the remaining 290 days, 8 mg. was given each dose (16 mg. per day). The injections were begun when the animals were 67 days of age.

B. Group II (12 rats) received a choline-deficient diet,‡ which was readily consumed, and in addition twice daily subcutaneous injections of serotonin were given in the same manner and amounts as to the animals in Group I. The injections were begun after the animals had been receiving the choline-deficient diet for 14 days; autopsy of one animal at that time showed fatty infiltration of the liver.

C. Group III (12 rats) served as a control group for Group I. Animals in this group received an ordinary diet and saline injections.

D. Group IV (11 rats) served as a control group for Group II. Animals in this group received a choline-deficient diet and saline injections.

II. Animals from each group were killed at varying intervals; in addition, some animals died unexpectedly during the experiment, and these will be discussed later. After 353 days of serotonin administration the studies were concluded and surviving animals were killed.

III. Each animal was autopsied as soon as possible after death, and in the majority of cases the heart was removed and placed in fixative while the animal was deeply etherized. Fixation was in Zenker's and 10% neutral formalin solutions; the stains used were hematoxylin and eosin, phloxine methylene blue, and Verhoeff's elastica stain. The organs examined microscopically were the heart and its valves, the lungs, the spleen, the pancreas, the liver, both kidneys, the adrenals, the esophagus, the stomach, the duodenum, the ileum, and descending colon, the tip of the tail, the tongue, the skin at the site of the injections, the eyes, and the brain.

Results

The morphologic findings may be briefly summarized as follows. Lesions were found in the tail, the extremities, the skin, the kidneys, and the eyes. These changes were necrosis of the tip of the tail and of the digits, tubular and occasionally cortical necrosis of the kidneys, dermal fibrosis and opacities of the lenses of the eyes. Production of fatty liver in animals receiving serotonin appeared to predispose to tubular and cortical necrosis of the kidneys, but serotonin neither enhanced nor protected against the hepatic changes that resulted from the choline-deficient diet.

Tables 1 and 2 summarize the administration of serotonin and the choline-deficient diet to the test animals and indicate the organs in which lesions were found. Of 12 rats given a choline-deficient diet and receiving serotonin, 7 died during the course of the experiment; all of these had renal lesions of tubular necrosis, generally with calcareous deposits within dilated tubular

* Purchased from the Charles River Breeding Laboratories, Cambridge, Mass.

† Supplied by George Berryman, M.D., of Abbott Laboratories, North Chicago, Ill.; by Merrill Speeter, Ph.D., of The Upjohn Company, Kalamazoo, Mich., purchased from the California Foundation for Biochemical Research, Los Angeles, and supplied by Vismara Terapeutici, through the California Foundation for Biochemical Research.

‡ Composition of the diet was as follows: arachin 12%, gelatin 6%, casein 3%, fibrin 1%, beef fat 10%, corn oil 2%, cod liver oil concentrate 0.015%, cystine 0.5%, Celluflour (cellulose flour) 2%, sucrose 57.5%, salts 5%, and vitamin powder 1%. Composition of the salts was as follows per 100 gm.: calcium lactate 35.0 gm., calcium carbonate 5.06 gm., $Ca(H_2PO_4) \cdot H_2O$ 14.60 gm., K_2HPO_4 6.46 gm., $NaH_2PO \cdot H_2O$ 18.76 gm., NaCl 9.34 gm., $MgSO_4 \cdot 4\ H_2O$ 0.33 gm., $ZnSO_4 \cdot 7\ H_2O$ 0.035 gm., $CuSO_4 \cdot 3\ H_2O$ 0.039 gm., KI 0.00039 gm.; composition of the vitamin powder was as follows per 1000 gm.: thiamine 500 mg., riboflavin 250 mg., pyridoxine 200 mg., calcium pantothenate 1 gm., nicotinic acid 1 gm., powdered sugar 997.06 gm.

TABLE 1.—*Animals Receiving Subcutaneous Injections of Serotonin Creatinine Sulfate (5-Hydroxytryptamine) and a Regular Diet*

Animal No.	Age at Death, Days	Weight at Death, Gm.	Serotonin		Site of Lesions
			Total Dose, Mg.	Administration, Total Days	
5	107	170	926	71	Skin, kidneys
12	230	165	2,870	193	Tail, eyes, skin, kidneys
17	232	209	2,906	195	Tail, eyes, skin, kidneys
19	270	185	3,514	233	Tail, skin, kidneys
24	379	275	5,258	342	Tail, digits, skin, kidneys
26	386	274	5,370	349	Tail, skin, kidneys
27	389	251	5,418	352	Tail, skin, kidneys
28	389	345	5,258	342	Tail, skin, kidneys
29	389	345	5,258	342	Tail, skin, kidneys
30	389	254	5,418	352	Tail, skin, kidneys
31	390	247	5,434	353	Tail, skin, kidneys

lumens; 1 died of pneumonia. The factor of early death is further brought out by considering that of nine animals that received serotonin for approximately five months or longer, seven died unexpectedly. By comparison, of 11 animals receiving serotonin but given an ordinary diet, only 1 animal died unexpectedly, and this animal had slight renal lesions. Of the 23 control animals, 12 given an ordinary diet and 11 given a choline-deficient diet, none died unexpectedly. None of the animals receiving serotonin developed diarrhea; the stools were soft formed pellets; however, hyperpnea and weakness were observed for approximately an hour following serotonin administration, and erythema of the ears and skin was noted for approximately the same period of time.

In more detail, the gross and histologic observations were as follows.

Heart.—The aortic, mitral, pulmonic, and tricuspid valves were similar in test and control animals. Small localized fibrous thickenings of the valve cusps were frequently noted in both test and control animals (Figs. 1 to 3). No increase in tissue mast cells [7] was noted in test animals; mast cells were normally found in the myocardium and along serosal surfaces. There were no myocardial or endocardial lesions.

Lungs.—The pulmonary parenchyma was similar in test and control animals; small arteries and arterioles in animals receiving serotonin were not more pronounced, and there was no discernible degree of thickening of the muscle walls when compared with control animals.

Spleen.—Lymphoid follicles were prominent in both test and control animals, and all animals had abundant extracellular hemosiderin pigment throughout the pulp tissue.

Liver.—The livers of animals given an ordinary diet were normal. The livers of animals on a choline-deficient, high-fat, high-calorie diet were similar in animals that received serotonin and in those that did not receive the drug. Fatty infiltration progressed to a well-marked cirrhosis after

TABLE 2.—*Animals Receiving Subcutaneous Injections of Serotonin Creatinine Sulfate (5-Hydroxytryptamine) and a Choline-Deficient Diet*

Animal No.	Age at Death, Days	Weight at Death, Gm.	Diet, Total Days	Serotonin		Site of Lesions
				Total Dose, Mg.	Administration, Total Days	
2	79	175	26	56	20	Liver, kidneys, skin
8	112	185	89	998	76	Liver, kidneys, skin
9	154	230	131	1,670	118	Liver, tail, kidneys, skin
11	182	140	139	2,102	145	Liver, tail, kidneys, eyes, skin
13	232	179	209	2,906	195	Liver, tail, kidneys, eyes, skin
14	232	179	209	2,906	195	Liver, tail, kidneys, skin
18	267	184	244	3,466	230	Liver, tail, kidneys, skin
20	291	210	268	3,690	244	Liver, tail, kidneys, skin
21	313	200	290	4,042	266	Liver, tail, kidneys, skin
22	339	178	316	4,618	302	Liver, tail, kidneys, skin
23	373	176	350	5,162	336	Kidneys, tail, eyes, skin
25	379	170	356	5,258	342	Liver, tail, kidneys, skin

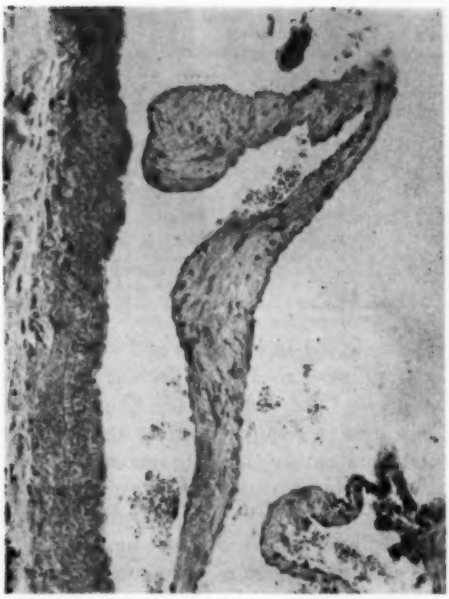

Fig. 1.—Pulmonic valve cusp of a rat that received 5434 mg. of serotonin creatinine sulfate in 353 days. Note thickenings of valve cusp. Hematoxylin and eosin; reduced 11% from mag. × 300.

Fig. 2.—Pulmonic valve cusp of a control rat that received a choline-deficient diet only for 356 days. Note similar thickenings of cusp. Hematoxylin and eosin; reduced 11% from mag. × 400.

Fig. 3.—High magnification of valve cusp shown in Figure 2. Note vacuoles within cusp. Hematoxylin and eosin; reduced 11% from mag. × 700.

approximately four months (Fig. 4) in 65% of the test animals, with fibrosis and marked fibroblastic scarring, with regeneration, and with fat. So-called "Mallory bodies," or "alcoholic hyaline," was not found.[8] The occurrence of fatty liver and the development of cirrhosis was related to the amount of fat and calories included in the diet as well as to the lack of choline.

Kidneys.—Lesions of the kidneys were found in all of 12 rats receiving serotonin and choline-deficient diet. These consisted of tubular necrosis with calcification (Figs. 5 and 6) and cortical necrosis. Of 11 animals receiving only a choline-deficient diet, 7 had occasional lesions consisting of tubular necrosis and calcification, but in these animals the lesions were present only in rare tubules (Fig. 7). In 11 animals given an ordinary diet and receiving serotonin, different renal lesions were found. These were tubular necrosis, regeneration, and dilation, with fibrosis of wedge-shaped areas of cor-

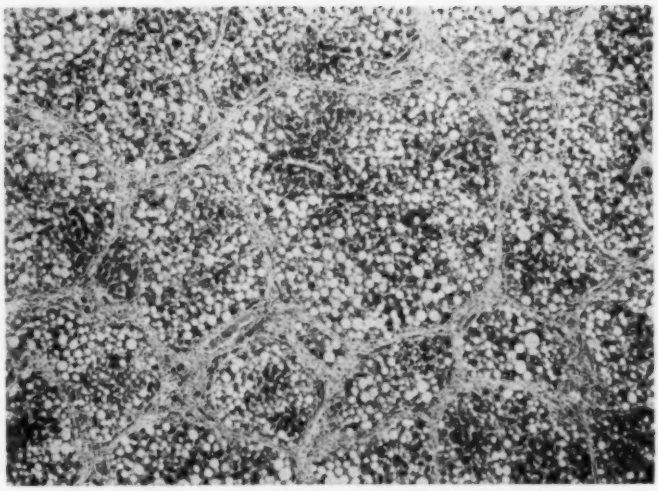

Fig. 4.—Cirrhotic liver of a rat that received 5258 mg. of serotonin creatinine sulfate in 342 days plus a choline-deficient diet for 356 days. Hematoxylin and eosin; reduced 32% from mag. × 800.

tex. Twelve control animals given an ordinary diet did not have renal lesions.

Adrenals.—Adrenal lesions were not found in animals receiving serotonin or in animals given a choline-deficient diet.

Gastrointestinal Tract.—Ulcerations or other lesions did not occur in test or control animals [2]; the mucosa was intact; mast cells were not more prominent in the gastrointestinal tracts of test animals. It is of interest, however, that Haverback and Bogdanski [9] succeeded in producing gastric mucosal ulcerations in rats by use of the serotonin precursor, 5-hydroxytryptophan.

Tail.—Among animals receiving serotonin, 20 had necrosis of the tip of the tail. In three animals, the necrosis was sufficiently extensive to have resulted in loss of nearly the entire tail. In the majority of animals, however, only the terminal two to six cm. was involved. The process consisted first of loss of the skin at the tip of the tail and progressed to loss of bone. In all but two animals healing of the skin over the denuded tip occurred.

Skin.—At the site of serotonin injections, scarring resulted, and accumulations of mast cells were observed in the dermis;

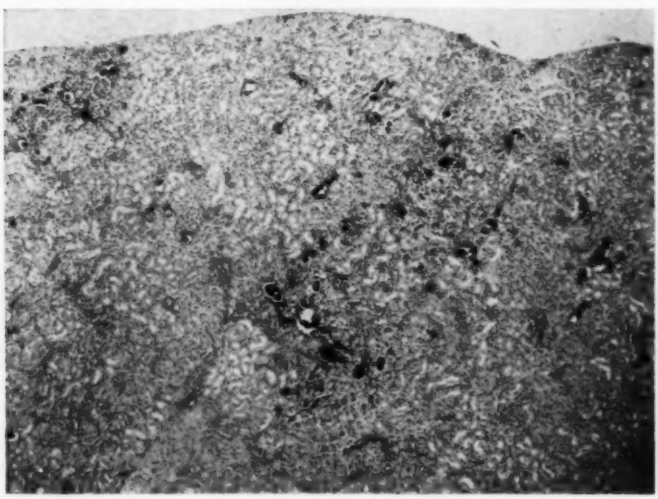

Fig. 5.—Renal cortex of a rat that received 3466 mg. of serotonin creatinine sulfate in 230 days plus a choline-deficient diet for 244 days. Note extensive tubular necrosis with calcareous deposits, other swollen and pale-staining tubules, interstitial mononuclear-cell infiltrations, and a small area of cortical atrophy. Hematoxylin and eosin; reduced 32% from mag. × 80.

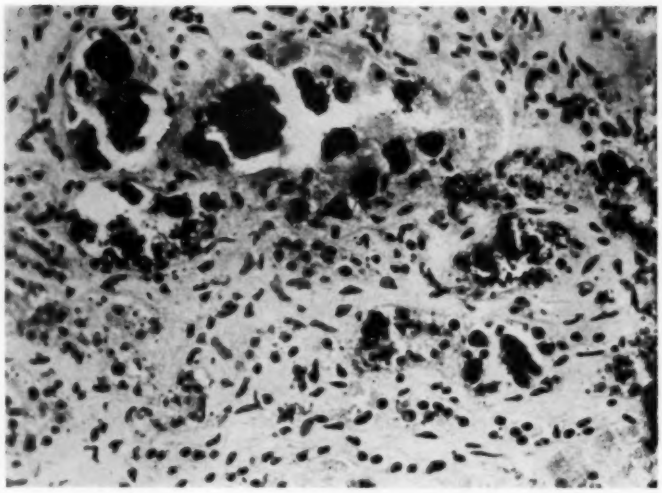

Fig. 6.—High-power magnification of kidney shown in Figure 5, showing tubular necrosis, calcareous deposits within tubules, and interstitial mononuclear-cell infiltrations. Hematoxylin and eosin; reduced 32% from mag. × 700.

but the mast cells were similar in animals receiving only saline control injections.

Tongue.—No constant lesions ascribable to serotonin were observed; animals receiving serotonin frequently had dilatation of veins and capillaries within the muscle bundles, and fat globules were common in animals receiving a choline-deficient diet.

Joints.—In one animal swelling and tumor of the terminal joint of a hindleg occurred, but this was not considered to be related to serotonin administration.

Pancreas.—No lesions were found.

Brain.—No lesions or vascular changes were found.

Lens.—In 6 of 23 animals receiving serotonin, opacities resulted. By comparison, none of the 23 control animals developed opacities.

Comment

In the present study, heart valve lesions did not result from the long-term subcutaneous administration of serotonin, de-

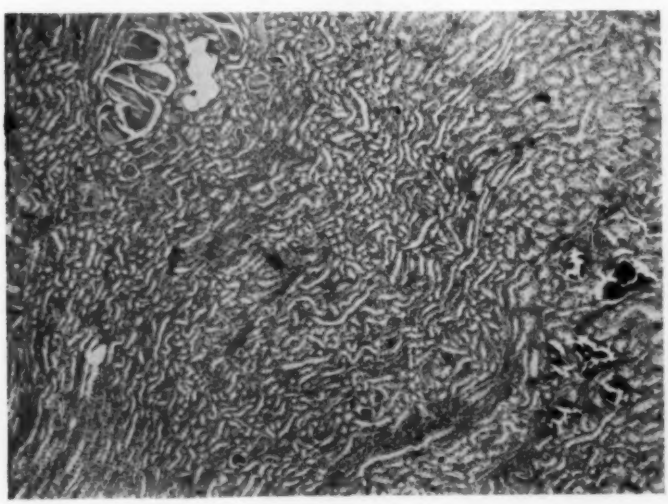

Fig. 7.—Renal cortex and medulla of a rat that received only a choline-deficient diet for 353 days, showing scattered areas with dilated tubules containing precipitates and other areas with tubular necrosis and calcareous deposits. Hematoxylin and eosin; reduced 32% from mag. × 80.

spite the presence of a fatty, and later a cirrhotic, liver. There was an increased incidence and severity of renal tubular necrosis in animals receiving serotonin in addition to a choline-deficient diet, which may have been due to an ischemic effect of serotonin. It has previously been shown that rats given a choline-deficient diet develop tubular necrosis if they are fed the diet while young and actively growing. The pathogenesis of the lesions had been explained [10] as the result of ischemia of small vessels that normally course between renal tubular epithelial cells. Compromise of these vessels is thought to occur as tubular epithelial cells fill with fat, become swollen, and compress the small vessels. However, older animals such as those used in the present experiment have been found to not ordinarily develop renal lesions when given a choline-deficient diet. In older animals the degree of fat-overloading of the tubular cells may not be as marked as in young animals, perhaps related in turn to differences in the phospholipid transport mechanism of fat and to the content of magnesium.[11] The occurrence of extensive tubular lesions in the older rats in the present study may have been due to an ischemic effect of serotonin, which, added to an ischemic effect on tubular vessels that occurs with a choline-deficient diet, was sufficient to cause a necrosis of the type seen in weanling animals that are given a choline-deficient diet alone. At the site of serotonin injections there was an unusual or abnormal fibroblastic process and the skin and subcutaneous tissue of animals receiving serotonin differed from that of animals receiving only saline control injections.

The failure to reproduce cardiac lesions by serotonin administration may have one or more of several explanations. Among these are the test animal used, the route of administration of the drug, the need for additional demands on the heart such as cardiac failure, and the inability of serotonin or the employed form of serotonin to bring about heart valve changes. Tachyphylaxis did not appear to be the explanation, since injections were discontinued for a two-week period in a few animals, and when injections were resumed, these animals did not suffer ill effects and, in fact, tolerated the injections as well as did the remaining animals. The efficacy of the form of serotonin was tested in additional animals, including dogs, rats, and mice, in which it produced profound physiologic changes; it produced death in rats and mice.

It is noteworthy that serotonin is but one substance in a metabolic pathway of tryptophan,[12] and it is conceivable that other substances in this pathway may be responsible for morphologic alterations. For example, 5-hydroxytryptophan, a serotonin precursor, has been shown upon injection to be converted by a specific decarboxylase to serotonin,[13] and 5-hydroxytryptophan could be the responsible agent in the carcinoid syndrome. The conversion of 5-hydroxytryptophan to 5-hydroxytryptamine and 5-hydroxyindolacetic acid in vivo would probably result in the formation of much larger quantities of serotonin in body tissues than it is possible to attain in an experiment in which serotonin is introduced, since injected serotonin is rapidly deactivated by the enzyme monoamine oxidase, which is normally present in many tissues. Further, more remote possibilities are that there are as yet undiscovered products in the metabolic pathway whereby tryptophan is converted to 5-hydroxyindolacetic acid and that there are genetic factors that render some persons susceptible to cardiac damage from serotonin. In considering the ways in which serotonin might affect the hearts of susceptible persons, the known vasoconstrictive action of serotonin, especially upon small terminal arterioles, may be invoked; ischemia of the heart and valves may result in fibrous-tissue proliferation within the endocardium. Observations in humans with cardiac valvular lesions of the carcinoid syndrome, in whom the earliest heart valve changes appeared to have been

the formation of fibrous tissue within the endocardium,[15] are consistent with this hypothesis. In a consideration of the use of serotonin as a therapeutic agent, the present experiment suggests a wide range of safety insofar as morphologic effects are concerned. The high and unphysiologic doses used in rats in this experiment would probably not be attained in therapeutic use in humans.

Summary and Conclusions

Serotonin creatinine sulfate (5-hydroxytryptamine) was administered subcutaneously to 23 rats in a dosage of 16 mg. per day for periods of up to 353 consecutive days. The largest total amount received by any one animal was 5434 mg.

Morphologic studies were made of the heart and its valves, the lungs, the spleen, the pancreas, the liver, both kidneys, the adrenals, the esophagus, the stomach, the duodenum, the ileum and descending colon, the tip of the tail, the tongue, the skin at the site of injections, the eyes, and the brain.

The morphologic lesions that resulted in test animals consisted of dermal fibrosis, necrosis of the tip of the tail, necrosis of the tips of the digits, tubular and cortical necrosis of the kidneys, and opacities of the eyes. These changes may all have been due to a vasoconstrictive action of serotonin.

Lesions of the heart valves analogous to those found in humans with the carcinoid syndrome did not occur, nor were ulcers of the gastrointestinal tract found. Increased numbers of tissue mast cells were not observed in the organs of animals receiving serotonin.

Fatty liver leading to cirrhosis was produced with a choline-deficient, high-fat, high-calorie diet in 12 of the rats receiving serotonin. Serotonin did not protect against or enhance the hepatic changes resulting from the choline-deficient diet. However, renal changes consisting of tubular necrosis appeared more frequently, earlier, and more extensively in the animals receiving a choline-deficient diet plus serotonin than in animals receiving only a choline-deficient diet.

It is suggested that the morphologic effects of chronically administered serotonin may be due only to its vasoconstrictive actions.

Histological preparations were made by Louise Mertz, A.B., and photography was by Mr. Leo Goodman.

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Pathologic Effects of Antimetabolites

II. Convulsions and Brain Lesions Caused by Methionine Sulfoximine, and Their Variation with Genotype

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Methionine sulfoximine, an analogue of methionine that produces fits in mammals, has been used to study the biochemical aspects of epilepsy. Tower and Elliott [1,2] found that it inhibits the formation of bound acetylcholine in brain slices and that the encephalographic disturbances it produces in laboratory mammals closely resemble those seen in some forms of human epilepsy. Misani and Reiner,[3] as well as others,[4,5] isolated methionine sulfoximine (MS) from flour bleached (agenized) with nitrogen trichloride and synthesized it. Such flour had been known earlier to induce seizures in dogs. Reiner also showed that methionine could prevent the MS fits, and Tower and Elliott showed that both methionine and glutamine counteracted its effect on the synthesis of bound acetylcholine in brain slices.

In order to study the pathologic effects of MS and learn more about its effects on brain metabolism in vivo two sets of experiments were carried out. In the first set MS was given to several kinds of mice and to rats, in which it always produced convulsions. Histologic studies of survivors showed that some degree of brain damage resulted in virtually all but C/57 Black 6 mice. When methionine was given before the MS it prevented both seizures and lesions. Because the damage varied considerably and was absent in C/57 mice despite convulsions, a second set of experiments was carried out to sharpen the picture if possible. These consisted of a comparison between C/57 Black 6 and DBA/2 mice in respect to their responses to MS. DBA/2 mice have a genetically determined "inborn error of brain metabolism," which expresses itself as a susceptibility to seizure induced by noise or by epileptogenic drugs including MS. This error, or constellation of errors, is present in a number of strains of mice, most strongly developed in DBA/2, a little less in DBA/1, and virtually not at all in C/57.[6,7] Abood and Gerard [8] characterized the error biochemically, with the use of DBA/1 mice, as a lag in the development of several of the components that maintain normal oxidative phosphorylating processes in the forebrain, and found this lag to be most pronounced between the 25th and 45th days of life, after which the faulty development seemed to catch up gradually.

The use of these two genotypes, DBA/2 and C/57, did sharpen the pathologic picture considerably, DBA/2 mice quite consistently showing severe brain damage after MS, with a number of interesting features. This paper will describe the two sets of experiments just outlined and discuss some of their implications against a background of previous studies of the effects of other inhibitors.

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Dr. L. Reiner, Research Director of Wallace and Tiernan Products, Belleville, N. J., made the methionine sulfoximine (Lot 76-249-283) used in these experiments, and Dr. Willard J. Johnson, Biochemical Research Director of Frank W. Hornor, Ltd., Montreal, made the 6-aminonicotinamide used in the preliminary experiments referred to.

Experiments

The general method was usually to give animals a sublethal dose of MS then kill the survivors the next day for pathologic study. A dose lethal for about 50% of the animals within 24 hours (LD_{50}) was most used. Trials showed that convulsions be-

gan in a few hours, and if death did not occur shortly the animal would usually live on, convulsing intermittently, and be stuporous for some 24 hours or longer. Death might occur at any time, but survival with "recovery" was possible in 24 to 48 hours. The animals whose pathologic examinations make up this report then represent roughly half of the animals given MS.

In the first set of experiments the design was to determine whether MS would damage the brain generally in mice and rats without special regard to genotype and whether this could be prevented by methionine. The experiments were conducted over a two-year period, during which the effects on several groups of animals were explored and the mode of administration of the drug was varied somewhat. Specifically, the animals that survived in these groups to be autopsied were seven albino rats (Wistar stock), seven BAF/1, nine C/57 Black 6, six Swiss albino, and four mice of an inbred black and tan-yellow cross. The rats, young male and female adults, weighed 160 to 250 gm. The dose to the rats was 30 mg. per 100 gm., and it was 4 to 7 mg. per 30 gm. for mice. The MS was given in aqueous solution or in commercial Wesson (vegetable) Oil suspension, intraperitoneally or subcutaneously, respectively. All but the 9 C/57 mice and one black and tan mouse showed brain lesions which ranged from occasional necrotic neurons in the neocortex and striatum to more extensive destruction, sometimes with considerable swelling of the cytoplasm of neurons and an unusual bubbly vacuolation of the neuropil. These changes will be described fully under the DBA/2 experiments below. Except for a few necrotic cells in the lobule centers of the liver of one rat, no other lesions were seen.

To test the effect of methionine, two groups of eight mice (four black and tan-yellow and four Swiss albino in each) were given the nearly certainly lethal dose of 7 mg. of MS intraperitoneally. One group, however, received 50 mg. of DL-methionine subcutaneously in 12 cc. of Wesson Oil an hour before the MS. The eight mice protected by methionine developed no visible effects from the MS, had no convulsions, and when autopsied 24 hours later showed no lesions. Their brains were examined in complete serial sections. The eight mice that did not receive methionine developed the usual convulsions, and five died in fits. The three survivors were killed in 24 hours, and examination showed characteristic cerebral damage.

This first series of experiments demonstrated that MS generally produced some degree of neuron and neuropil damage in the forebrain of mice and rats as an accompaniment of the convulsions with the exception that C/57 mice showed no lesions. The routes and modes of administration of the drug did not seem to be a major factor in variability of response. Methionine prevented the lesions and the convulsions, demonstrating a metabolite-antimetabolite antagonism, although it did not reveal where or how it took place.*

In the second series of experiments the effects of MS were narrowed down to a study of two strains of mice selected for the reasons noted, and the forebrain was the chief object of study. They were carried out over a four-month period on DBA/2 and C/57 Black 6 mice, males and females, 7 weeks to 3 months old. This age is past the critical period when DBA mice are ordinarily most susceptible to audiogenic seizures. These mice were obtained from the Jackson Memorial Laboratory at Bar Harbor, Maine, during the period of experimentation. As in previous experiments the animals were kept in a laboratory at about 72 to 80 F and fed Purina Lab Chow and water freely. Sixteen DBA/2 mice out of 30 given MS and 11 C/57 mice out of 17 given MS survived to be studied histologically. They received 5 mg. of MS per 30 gm. of body weight subcutaneously or intraperitoneally, and convulsions developed quite regularly in about four hours. Most of the animals were killed 16 to 24 hours after the MS, but four were killed in 10 hours; two, at 30, and three, at 48 hours in order to observe the progress of whatever lesions might develop. Complete autopsies of all organs were done in 19 animals, while in the remaining 7 only the brain was studied microscopically. Sections almost always were made of the eyes; pituitary; temporal bone (ear); neck organs, with thyroid and rarely parathyroid; skin; salivary gland; tongue; thymus; heart; lung; liver; spleen; pancreas adrenal; urinogenital tract organs; stomach; large and small bowel; diaphragm; rib with muscle; one or more levels of spine, with bone, muscle, and nervous elements; sciatic nerve with muscle. Fixation was usually in Bouin's fluid made with 10% formalin. (The brains of three additional MS-treated DBA/2 animals were prepared for Cajal and Golgi silver impregnations to study the damage in the neuropil.) The brains were examined by multiple sections of the front brain (about 200 to 400 sections), but only a few sections of the hindbrain and cerebellum were made. They were stained with hematoxylin and eosin, cresyl violet, phosphotungstic acid hematoxylin, and occasionally Bodian protargol.

A few other experiments were run to check the effects of methionine or glutamine in MS poisoning. When 50 mg. of DL-methionine was given

* At the same time, a series of mice were given L-glutamic acid in oil prior to MS. The dose, 100 mg., is about the maximum tolerated in the circumstances. It prevented neither convulsions nor brain lesions.

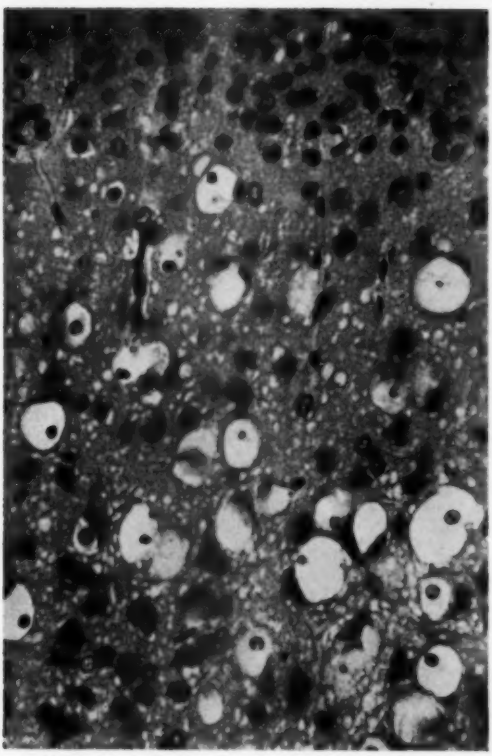

Fig. 1.—Acute vacuolar change in neuron bodies and neuropil in cingular cortex of DBA/2 mouse 17 hours after MS. Phosphotungstic acid hematoxylin; about × 800.

an hour before 5 mg. to 7 mg. of MS in DBA/2 mice, it prevented convulsions and brain lesions. Given 15 hours in advance, it was ineffectual, and if it was given just before the onset of convulsions, it had no protective effect. Glutamine in single 25, 50, or 100 mg. doses was given an hour before MS, but these failed to protect against convulsions. Possibly the glutamine could be given more effectively and protective results obtained, but it seems on a basis of limited experiments that glutamine is at least inferior to methionine in counteracting the effects of MS.

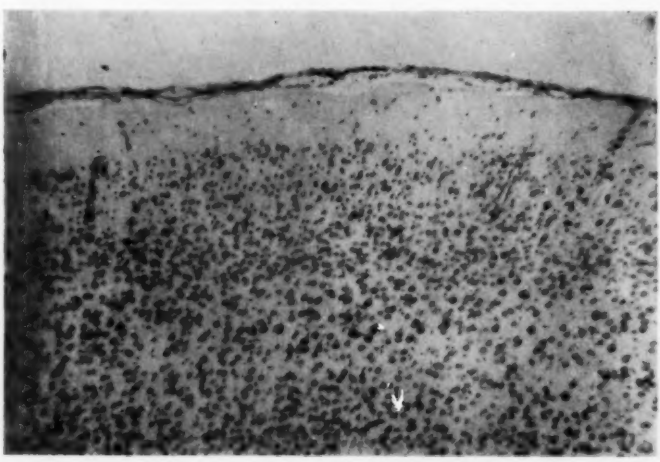

Fig. 2.—Acute necrosis of parietal cortical neurons, especially Lamina II and outer Lamina III, in a DBA/2 mouse 37 hours after MS. Cresyl violet; reduced about 15% from mag. × 200.

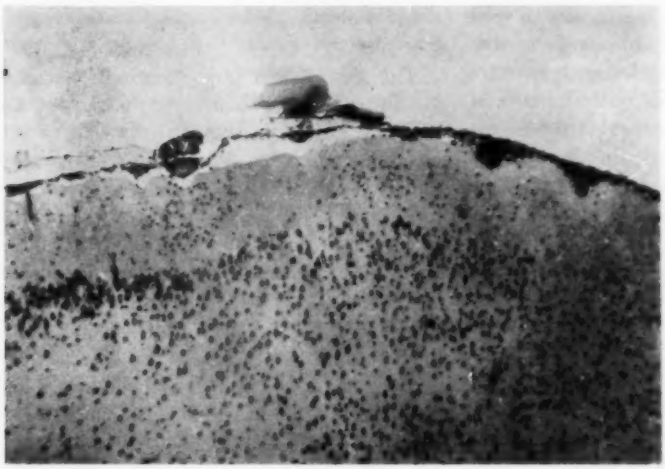

Fig. 3.—Polymorphonuclear leukocytes in meninges and in neuropil of Lamina I at junction of neocortex and entorhinal cortex. DBA/2 mouse 22 hours after MS. Cresyl violet; reduced about 15% from mag. × 200.

Results of Experiments

In the 15 DBA/2 and 11 C/57 mice studied for the effects of MS, only the DBA/2 mice showed brain lesions (Figures), and no other distinctive tissue changes were found in either series. (Some adrenal changes in DBA/2 mice are noted later.) All of the DBA/2 mice showed lesions of the forebrain, moderately severe to very severe in 12 and mild in three. There were three principal characteristics of the lesions: necrosis of some neurons, extraordinary vacuolar swelling of the cytoplasm of others, and a fine bubbly, vacuolar swelling and degeneration of neuropil. A fourth feature of interest was the prominent appearance of polymorphonuclear leukocytes in the superficial brain vessels and in many capillaries in Lamina I. It was notable in early stages and usually subsided later. The leukocytes fairly frequently infiltrated the neuropil. Leukocytes were not evident in other tissues or in uninvolved brain and did not appear to reflect vascular stasis. The swelling of neurons and neuropil predominated in animals killed 10 to 20 hours after MS, while necrosis was more evident in those allowed to live longer, but distinctly necrotic cells were evident in 10 hours. Cytoplasmic swelling may have been a prelude to morpho-

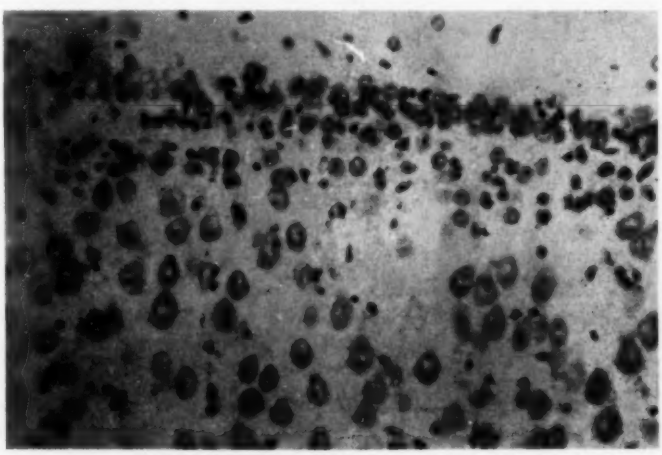

Fig. 4.—Acute necrosis of cortical neurons, especially Lamina II, in cingular cortex. DBA/2 mouse 36 hours after MS. Cresyl violet; reduced about 15% from mag. × 800.

logically evident necrosis, but as will be seen it probably did not invariably progress that way. The regions involved by necrosis were those of the neocortex, especially Lamina II and outer Lamina III throughout the extent of the neocortex, although the dorsolateral frontal and parietal areas were usually the hardest hit. The cingular cortex was also usually heavily involved; the entorhinal cortex was infrequently involved, and yet the amygdaloid nuclei were often damaged. Cell bodies in the striatum, principally forward in the caudate-putamen and less in the globus pallidus, were nearly as often and severely affected as the cortex. The pyramidal layer of the hippocampus, especially those specimens from the dorsal and anterior parts, frequently showed involvement, and sometimes the cells of the fascia dentata also suffered. In six animals that had severe cortical, striatal, and hippocampal damage there was also moderate to severe involvement (including both dead and vacuolated neurons) of most of the anterior, ventral, and some central thalamic nuclei, as well as the anterior hypothalamic area and subthalamic nuclei posteriorly. In one of these animals there was virtually no part of the cortex and interbrain, anterior to the midbrain colliculi, that did not have a few to large numbers of necrotic neurons, and yet it had lived 20 hours.

Necrotic neurons were not different in appearance from those seen after some other acute metabolic inhibitions, such as anoxia or hypoglycemia, whether in laboratory mammals or man. Recognizable as early as 10 hours, they were characteristic by 15 hours, having a much reduced volume of cytoplasm and nucleus. The cytoplasm was usually shrunken but indistinct and often frayed in outline, usually eosinophilic but sometimes very pale. The nucleus was similarly indistinct in outline, but it was either a compact basophilic smudge or represented by disintegrating granules of chromatin. (In contrast, artifactually shrunken cells, which are sometimes hard to distinguish from those with early necrobiotic changes, retain a sharp cell border and nuclear membrane.)

The cytoplasmic and neuropil vacuolar swelling were notable because they have been virtually absent in experiments with other metabolic inhibitors. Most fully developed in DBA/2 mice, they were characteristically present but milder in many of the animals in the first experiments. The neuropil of Lamina I of the cortex, where dendritic processes and their synapses constitute most of what is there, and corresponding dendritic regions of the hippocampus were particularly affected in the animals killed 10 to 24 hours after MS. The change was characterized by a fine diffuse vacuolated or bubbly appearance, mottled but extensively distributed. It also involved neuropil in outer Lamina II and less in Lamina III, and, though not as extensive, it was sometimes well developed in deeper cortical layers. The middle cingular cortical layers were frequently involved. The striatum and diencephalon showed considerably less. The change could not be entirely accounted for by saying that it was disintegration of the dendritic processes of necrotic cell bodies, for the altered neuropil infiltrated by leukocytes often appeared adjacent to regions in which little or no cell death was yet evident. It seemed to represent selective reaction to injury in neuropil regions. In animals killed 36 to 48 hours after the MS was given, the outer neuropil was more acidophilic than usual and its fibrillar character, indistinct, though not as vacuolated as in earlier stages. Silver impregnations of brains of the three additional mice killed 36 hours after MS showed nearly complete disintegration of the fibers in Lamina I and of many in Lamina II. However, the cell bodies in Lamina II and outer Lamina III were nearly all dead in these particular animals, and much of it could have been and clearly was secondary to the death of these cell bodies. Notwithstanding, the terminal bushels and branches of afferent dendrites from lower levels were similarly involved in Lamina I.

Cytoplasmic vacuolation of the bodies of neurons was most prominent in certain cortical regions but also involved basal structures and hippocampus and in severe cases was widespread. Neurons in Lamina II, III, and V seemed to be conspicuously affected in the dorsolateral neocortical regions, however. The cytoplasm of the cell became almost a complete round vacuole three or four times the normal cell diameter, sometimes with a wisp of cytoplasm and often with the nucleus of a satellite cell. The nucleus of the neuron itself, when it appeared in the section (sections were cut 8μ thick), was flattened along one margin. Glial cells rarely showed the vacuolated cytoplasm, but astrocyte nuclei were sometimes swollen. The nucleus in many of the vacuolated neurons was so well preserved and relatively normal-looking that it was difficult to think that this swelling was invariably a step toward necrosis. That it could represent a still reversible state of damage is suggested by the relatively infrequent dead neurons in Layer V in mice killed late, compared to the number of vacuolated neurons in this layer in the earlier hours after MS.

Although destructive lesions did not appear in other organs, the adrenals of the DBA and C/57 mice were enlarged at autopsy to nearly twice that of normals. Microscopically they showed more than usual mitotic figures in the outer glomerulosa zone.

Convulsions

Although the approximate LD_{50} dose of MS was the same for both the DBA/2 and C/57 mice, it is interesting to note that the DBA/2 mice not only showed a different pathologic response in the brain from C/57's but that the convulsive pattern was also somewhat different. The time of onset of overt convulsions was very close to four hours in virtually all animals. It was preceded by a half-hour stage of slight restlessness and a tendency for the animals to extend their extremities and show a fine generalized tremor when stimulated. The animals were studied together in groups of three to six, and so the first animal to convulse undoubtedly triggered the others, but they were ready to convulse anyhow. After the first seizure, DBA/2 mice averaged some five or six convulsions per hour, with some of them having as many as four consecutive convulsions in a 15-minute span. The C/57 Black 6 mice had far fewer convulsions during the same length of time and tended to stand with limbs, head, and ears extended and tremorous, especially if they were handled. Many C/57 animals died immediately after their first or second convulsion, whereas DBA/2's usually took longer. Most convulsions in both strains consisted of a totally uncontrolled running seizure, during which the animals ran and jumped into the sides and top of the cage. This lasted for approximately 20 seconds, leaving the animal breathing rapidly. Often the seizure terminated in a spasm in which the limbs were extended and the neck flexed, and at times this was preceded by clonic convulsions for a few seconds. The spasm lasted perhaps 5 to 10 seconds, when the animal relaxed or died.

It has been suggested that a vulnerable respiratory center might be the factor limiting survival in audiogenic seizures, because there is a period of apnea in the spastic stage of the fit and revival may follow artificial respiration.[7] In the experiments this apneic phase did not last long enough to be accountable for death in usual circumstances, and usually when such a seizure terminated in death the animal's heart beat and respiration simply stopped promptly. Nevertheless, this might still be a mechanism of death, because it is well known that seizures exhaust the energy-rich phosphate stores of the brain with great rapidity. A brief period of apnea might be all that was needed. If this was the mechanism—apnea acting on an exhausted respiratory center—it would almost seem, paradoxically, that the C/57's were more susceptible.

Animals that were kept for 24, 36, or 48 hours often appeared moribund and felt cooler than normal, and yet when stimulated

they would often walk or crawl about and sometimes convulse. Some, however, were in extremis at the time of autopsy, but this did not seem to contribute to the severity of the lesions, because the C/57 mice showed none.

Comment

There are two aspects of these experiments that may be discussed. The first is the lesions themselves, for they have some interesting features. The second concerns the possible modes of action of MS in producing the lesions.

Lesions Caused by MS.—The neuronal cell body destruction in the neocortex and striatum caused by MS was similar to that seen in other experiments after acute asphyxia, insulin hypoglycemia, and inhibitors of cytochome oxidase,[9] but the involvement of thalamic and other diencephalic nuclei represented a difference. The extraordinary swelling of cell body cytoplasm was novel.

Especially interesting was the apparently selective involvement of the neuropil. This was evidenced by the fine vacuolar swelling and a variable infiltration by leukocytes into the vacuolated regions, especially Lamina I. Other places where neuropil is dense were fairly severely involved at times. This kind of change was not characteristic of the damage caused by a number of other metabolic inhibitors with which we have produced cerebral lesions (although it was encountered after 6-aminonicotinamide in a different pattern). How much of this degeneration in the early stages was in the ground substance and how much was cytoplasmic swelling of dendrites and axones and glial processes was difficult to assess. The polymorphonuclear leukocytic exudation into such regions in early stages, especially where the cell bodies corresponding to the damaged neuropil appeared intact, was taken to indicate primary irreversible breakdown of at least some components of the neuropil, either the fibers themselves or the intercellular ground substance. Intercellular and intracellular edema locally involving the cell processes could also have contributed to the picture, but there was no marked general edema.

There are other instances in which the processes of nerve cells can be selectively damaged without necessarily killing the whole cell, as, for example, in mechanical or metabolic injuries in peripheral nerves. Despite the fact that nerve fibers receive a flow of cytoplasm from the cell body,[10] they seem to differ metabolically from the cell body in several respects. In earlier experiments selective and acute destruction of myelinated fibers (mostly axons) in corpus callosum, optic nerve, and striatal bundles could be obtained by giving cyanide, azide, or malononitrile.[9] Whether these cytochrome oxidase inhibitors acted directly on these fibers or did the damage by killing the associated oligodendroglia, they illustrate that selective damage to cell processes can occur in the central nervous system. There seems to be no reason why dendrites should not also be selectively damaged. Further studies with silver stains in earlier stages of MS poisoning and the effects of other inhibitors on DBA/2 mice may establish this finding more firmly.

Mode of Action of MS.—MS is being used as a tool in biochemical, antimetabolite, genetic, and neurophysiologic research, and so continued information on its mode of action is desirable. Knowledge of the action of an antimetabolite, enzyme inhibitor, or any form of metabolic inhibition is usually obtained from a combination of approaches. Generally accepted notions about the special vulnerability of parts of the cerebral cortex to lack of oxygen or glucose are a composite built from observations of neurophysiologic, anatomic, pathologic, histochemical, and biochemical measurements. When an experimenter wants to build up such a picture of the effect of an inhibitor by observing the differential susceptibility of the various cells of the living organism to it, he needs to consider a number of variables. When a substance is given, many factors may conspire to distribute it unevenly in the organ-

ism and make its effect variable after it arrives at the cell.

Differences in blood supply and blood flow may deliver a substance, whether it is a noxa or a metabolite, to different regions at different rates and having delivered it may also clear it out at different rates if it is not disposed of locally. Tissue barriers like the blood-brain barrier may completely bar a substance from certain regions in one circumstance but admit it under another. For example, glutamine, a prime metabolite for the brain, passes the barrier, but glutamic acid as such cannot enter until it is converted to glutamine. The form of the substance which determines its absorption and mobility or whether it must be changed to another compound before it can act is a factor that may have to be considered. Assuming that many mobile substances do get widely and reasonably evenly disseminated, as there is reason to think the case often is, then comes the hardest problem, that of deciding how they produce their effects in the cell. Even when two substances have a similar biochemical action in vitro it cannot always be predicted that they will be handled the same way in vivo, granted that they are both delivered to the cell. Two examples from past studies [9,11,12] illustrate this. As mentioned before, cyanide, azide, and malononitrile (which liberates HCN in vivo) selectively damaged white matter, and this was thought to be correlated with the paucity of cytochrome oxidase in these regions. However, a number of other organic nitriles that yield HCN on hydrolysis have since failed to imitate these effects. Similarly, 3-acetyl pyridine and 6-aminonicotinamide,[13] both analogues of nicotinamide, produce different patterns of lesions in the living animal, and yet they show biochemical resemblances in respect to being incorporated into pyridine nucleotides, and their pathophysiologic effects are prevented by nicotinamide. Pyridine sulfonic acid, a third analogue, though lethal to experimental animals, produces no specific lesions when given in sublethal doses.

In view of these obstacles to interpreting the effects of metabolic inhibition in vivo, can anything be said about how MS produces convulsions and brain damage? Some correlations between the biochemical, physiologic, and pathologic effects can be attempted, although discrepancies remain. Glutamic acid and its amide, glutamine, are apparently very important to energy metabolism in the central nervous system, "glutamine acting as a store of metabolically inert glutamic acid, ketoglutaric acid and ammonia easily mobilized by enzymatic action—similar to the role of glycogen in carbohydrate metabolism." [14] MS inhibits the synthesis of glutamine from glutamic acid, which requires adenosine triphosphate, possibly by competing with glutamic acid in the reaction, and it strongly blocks a glutamyl transferase reaction.[15] This latter system provides a means for incorporating the glutamyl radical into polypeptide synthesis and may possibly be important in maintenance of brain protein. Glutamic acid is also important to the enzymic synthesis of acetylcholine,[14] but the metabolic steps concerned and their relation to MS, if any, are still unsolved.

The obvious way to explain the effects of MS would be to say that blocking glutamic acid metabolism right at the wellspring could have nothing but serious effects on the brain. Inhibition of oxidative energy-yielding processes in general are often expressed by convulsions, and in some cases the inhibition can be pushed to a sublethal point, where selective damage to nervous tissue occurs without death of the total organism. In other cases this differential effect is not obtainable because the lethal convulsive episodes are the limiting factor. It might be said that DBA/2's were most seriously affected because they already had an inborn defect in energy-yielding processes and the additional deficit caused by MS could only be disastrous. C/57's might be regarded as having escaped the tissue damage because they had a "normal" brain metabolism and convulsive death precluded larger doses of

MS. Undoubtedly this explains part of the picture, but it is based on the assumption that the DBA/2 animal is abnormal and the C/57 is normal. Normalcy is a highly relative matter from the genetic viewpoint. In respect to the oxidative phosphorylating mechanisms measured by Abood and Gerard,[8] it is probably reasonable to compare the DBA/2 forebrain against that of the C/57. When the rest of the animal is compared there are discrepancies. The lethal dose of MS (based arbitrarily on death during seizure in the first 24 hours) was the same for each strain of mice, and so there must be another difference between the two strains. Although it is not obvious why MS is lethal in these circumstances, the animals died in convulsions or immediately after the episode, and so death relates closely to the seizure. Thus, while DBA/2's differed from C/57's in being more susceptible to severe convulsions and destruction of brain tissue, the C/57's were in turn more sensitive to whatever the immediate lethal process was. (The other strains may possibly fall at points between.) A mechanism implied in an earlier paragraph was that the cardiorespiratory centers in C/57's might have been more susceptible to the exhausting effect on energy stores of brain tissue by convulsions. However, MS is not limited to brain tissue in its metabolic effects, and so an extraneural component in the lethal process is not excluded.

Another possibility, at least as complex, needs to be explored. Elliott [17] has suggested that MS may interfere with transmethylating reactions in the synthesis of choline and thus affect acetylcholine metabolism. This would provide two sites of action for MS, one that might be reflected primarily in functional disturbances, while the other, through interference with glutamic acid metabolism, could inhibit function as well as lead to structural damage. These possibilities, however, still do not resolve all of the differences in response between C/57 and DBA/2 mice.

Summary

The pathologic changes and convulsions produced by methionine sulfoximine (MS) in several mouse genotypes and in rats were studied with emphasis on the contrasting patterns in DBA/2 and C/57 Black 6 mice. In DBA/2 mice destruction of forebrain neurons, especially neocortical but also diencephalic, and damage to neuropil were often severe, while in C/57 mice the lesions were absent. Convulsions occurred in both strains. Other animals showed intermediate degrees of damage.

The possible mode of action of MS is discussed. The contrasting effects may be largely explained by the fact that MS blocks the utilization of glutamic acid at a fundamental level, possibly by competing with the glutamic acid in some of its reactions, especially its conversion to glutamine. DBA/2 mice are presumed to have an inborn metabolic error in which oxidative phosphorylating mechanisms are deficient. The combination of this inborn defect coupled with the MS-induced interference with another energy-yielding pathway might account for the destructive damage in DBA/2 mice. Unsolved problems remain, however. One, methionine more effectively counteracts the effects of MS than glutamine, and the precise site of action of MS is still unknown. Another, C/57's escaped forebrain damage, but they were relatively more susceptible to the lethal process associated with the convulsive episodes than DBA/2's.

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Endocarditis and Glomerulonephritis in Dogs with Aortic Insufficiency

Production by Single Bacterial Inoculation and Effect of Cortisone

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In a previous study[1] it was shown that an infection resembling acute and subacute bacterial endocarditis could be induced readily in dogs with aortic insufficiency by multiple intravenous injections of cultures of Staphylococcus pyogenes var. aureus and Streptococcus mitis, respectively. The present study was undertaken to determine the effects of a single bacterial injection. The purpose was to simplify the method of producing endocarditis and to facilitate the evaluation of possible factors contributing to the increased susceptibility of such dogs to endocarditis.[2] Because some of the dogs developed a severe diffuse proliferative glomerulonephritis, which resembled somewhat that produced by an antigen-antibody reaction,[3] the effects of cortisone therapy were studied and are reported.

Materials and Methods

Aortic insufficiency was induced in 12 mongrel dogs of both sexes, weighing 10 to 15 kg., by perforating an aortic leaflet with a punch introduced via the ascending aorta.[4] The punches had cutting diameters of 3.9 and 4.9 mm. Ten dogs had a single defect, and one had two overlapping defects in the right coronary cusp, while the twelfth dog had a defect made in the left coronary cusp. Nine to twenty-one days after surgery, each dog received intravenously 1 cc. of a five-hour bacterial culture in beef-heart infusion broth containing 0.5% destrose. Seven dogs received S. mitis JH26 (Lancefield D), while five received S. pyogenes var. aureus; these organisms were used in the previous study.[1]

To study the effects of cortisone, 11 additional dogs with aortic insufficiency were given bacteria. Five received a single and one received two successive daily injections of cultures of S. mitis JH26, while four dogs received a single and one received five injections of S. pyogenes var. aureus. All 11 dogs received cortisone (Cortone, Merck & Co., Inc.) acetate, usually 100 mg per kilogram subcutaneously per day, beginning two to five days before injection of the bacteria until the day preceding the autopsy.

Blood cultures were taken before and at intervals after the bacterial inoculations. Blood cell counts and determinations of the hematocrits, sedimentation rates, and serum alkaline phosphatase values were also made. The latter was used to detect the development of infarcts.[5] A Statham transducer and direct recorder were used to obtain percutaneous femoral arterial pressure pulses. In general, femoral arterial pulse pressures ranged from 100 to 150 mm. of Hg. At autopsy, blood cultures were taken from the left ventricle. The heart and blocks of other organs were fixed in a 10% aqueous solution of buffered formalin (pH 7.0). Paraffin sections 6μ thick were prepared and stained routinely with azure eosin.

Results

All 12 dogs in the group which did not receive cortisone developed endocarditis. The blood cultures of all, except Dog 55 given S. mitis (Table), became positive. The dogs given staphylococci became acutely ill within 24 hours and died or were killed preterminally in 3 to 12 days. The course was less fulminating after an injection of

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National Institute of Arthritis and Metabolic Diseases (Drs. Highman and Altland) and Clinic of Surgery, National Heart Institute (Dr. Roshe), National Institutes of Health, Public Health Service, Department of Health, Education, and Welfare. Present address of Dr. Roshe: Department of Surgery, Indiana University Medical Center, Indianapolis.

Location and Grading of Lesions in Dogs with Aortic Insufficiency *

Dog †	Survival, Days	Mitral Valve ‡	Aortic Valve ‡	Sut. Line of Aorta ‡	Infarcts §		Proliferative Glomerulonephritis ‖	Remarks ¶
					Kidney	Spleen		
Dogs Given S. Pyogenes Var. Aureus								
48	3	++	++	+	P	P	+++	
53	4	++	++	++	P	P	++	
54	4	++	++	++	P	P	+++	
100	12	−	+	++	P	−	+++	Rupture of suture line
116	3	+	+	++	P	−	++	Rupture of suture line
Dogs Given S. Pyogenes Var. Aureus and Cortisone								
92	10	+	+	+	−	−	++	
93	6	−	−	++	−	P	+	Rupture of suture line
103	11	++	++	++	P	P	++++	
114	4	++	++	++	P	P	++++	
98	7	++	+	++	P	P	++++	
Dogs Given S. Mitis JH26								
47	8	++	++	+	P	P	+++	
52	7	++	++	+	P	P	++++	
55	10	+	++	−	P	−	+	
68	12	++	+	+	P	P	++	Rupture of chorda
105	20	++	++	+	P	P	++++	Perirenal abscess
81	55	+	−	+	−	−	+++	
97	203	−	−	−	−	−	+++	
Dogs Given S. Mitis JH26 and Cortisone								
83	9	−	−	+	−	−	++	
87	11	−	+	+	P	−	++	
88	11	−	+	+	P	−	+	
89	11	++	++	+	P	−	+	
91	10	++	+	+	P	P	++	
90	10	++	−	−	P	−	++	

* Given 1 cc. of a culture of S. pyogenes var. aureus or S. mitis JH26 intravenously with or without 100 mg. per kilogram of cortisone acetate subcutaneously daily.
† All dogs received a single bacterial injection except 90 and 98, which received 2 and 5 injections, respectively.
‡ ++ denotes vegetations containing demonstrable bacterial colonies, and + denotes inflammatory lesions with no demonstrable bacterial colonies.
§ P indicates infarcts present; −, no infarcts.
‖ See text for method of grading.
¶ Immediate or contributing cause of death is listed in the dogs that died; the others were killed.

S. mitis. One died in 12 days and showed massive pulmonary edema and mitral insufficiency due to rupture of one of the chordae tendineae to the anterior mitral leaflet. Another died in 20 days and showed severe endocarditis with a huge perirenal abscess. The other five did not appear acutely ill when killed (Table). Since survivors in our previous study [1] were killed within three weeks, the two dogs in this study observed for a longer period (Table) will be considered as a separate group.

The pathologic changes in the 10 dogs in this study that died or were killed within three weeks after the bacterial inoculation were similar to those reported in the dogs with aortic insufficiency given multiple injections of the same organisms.[1] All 10 dogs showed bacterial lesions (++ in Table) involving the mitral or aortic valve or the suture line of the aorta (Figs. 1 to 4). In addition, a few small superficial inflammatory foci were often seen in the tricuspid and occasionally in the pulmonary valve. The dogs given staphylococci showed extensive subendocardial hemorrhages and numerous hemorrhagic and suppurative lesions in nearly all organs. Such visceral

Fig. 1.—Heart of Dog 48, given staphylococci, showing bacterial vegetation along upper rim of surgical defect in right (middle) aortic cusp. The suture line in the aorta above showed no bacteria. Small vegetations are present on the auricular surface of the bisected anterior mitral leaflet in lower right portion of figure.

Fig. 2.—Anterior mitral leaflet of Dog 54, given staphylococci, showing extensive necrosis and numerous darkly staining bacterial colonies in distal half. Azure eosin; × 6.

lesions were less pronounced in the dogs which received streptococci, but the valvular vegetations were larger (Figs. 3 and 4).

Nearly all dogs developed infarcts in both the kidney (Fig. 5) and spleen and a diffuse proliferative glomerulonephritis (Fig. 6). The infarcts were evidenced during life by a sharp rise in serum alkaline phosphatase.[5] The glomerulonephritis was similar to the renal lesions associated with human endocarditis and previously produced in dogs.[1,6] It was characterized by an increase in cellularity of the glomeruli, with partial

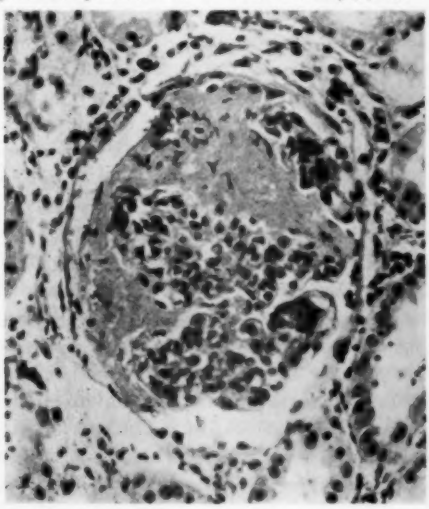
Fig. 3.—Heart of Dog 47, given Streptococcus mitis, showing large vegetation lying between and on the auricular surface of both mitral cusps.

Fig. 4.—Posterior mitral leaflet of Dog 47, showing large vegetation on auricular surface. Azure eosin; × 9.

Fig. 5.—Renal infarct in Dog 52, given streptococci.

obliteration of the capillary bed. The degree of cellular proliferation was estimated by counting cell nuclei in sections 6μ thick of 10 glomeruli taken at random in noninfarcted portions of the kidney. In general, the number of nuclei per glomerulus ranged between 80 and 125 in controls with or without aortic insufficiency which did not receive bacteria. Control dogs without aortic insufficiency given bacteria usually showed a normal range in cellularity of the glomeruli, but increased cellularity was noted occasionally after multiple bacterial injections. The lesions were graded 1+ (+ in Table); if the average number of nuclei per glomerulus ranged between 135 and 149; they were graded 2+ if between 150 and 174; they were graded 3+ if between 175 and 199,

Fig. 7.—Focal glomerulonephritis in Dog 66 given Streptococcus mitis. Azure eosin; × 250.



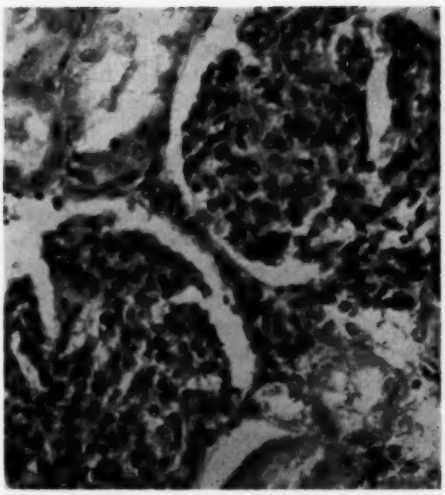


Fig. 6.—Proliferative glomerulonephritis in Dog 52. Azure eosin; × 312.

and 4+ if the number of nuclei was above 199. As shown in the Table, the severity of the glomerulonephritis tended to vary with the severity of the endocarditis and the duration of the infection. One dog (Dog 66) which received streptococci developed, in addition to the proliferative changes, a focal glomerulonephritis with hyaline changes in the tufts of occasional glomeruli (Fig. 7). These focal lesions were similar to the focal glomerular lesions associated with subacute bacterial endocarditis in man. Bacterial emboli were seen occasionally in renal capillaries, particularly in the medulla in dogs given staphylococci, but did not appear to be closely associated with the focal or proliferative glomerular changes.

Dogs 81 and 97 were observed for 55 and 203 days, respectively, for study of the cardiac and renal lesions in later stages of development. Their blood cultures became persistently negative 11 and 89 days, respectively, after the injection of streptococci, and left ventricular blood cultures taken at autopsy were negative. Neither dog developed a rise in serum alkaline phosphatase, and neither showed infarcts at autopsy. Both dogs showed slight valvular thickening, mainly involving the anterior mitral leaflet

and the rim of the surgical defect in the right aortic leaflet. In addition, Dog 97 showed an old rupture of one of the chordae from the anterior papillary muscle to the anterior mitral leaflet.

Microscopically, the thickened leaflets showed increased cellularity and a few scattered inflammatory cells, mainly lymphocytes. The anterior mitral leaflet of Dog 81 (55 days) showed a thick layer of vascularized loose cellular fibrous tissue along a portion of the auricular surface, with a few scattered hemosiderin-laden phagocytes; in one area, this tissue formed a projection suggesting an organized vegetation (Fig. 8). Section of the ends of the ruptured chorda in Dog 97 (203 days) revealed several small cellular foci showing central necrosis; the tip of the subjacent papillary muscle showed subtotal replacement of muscle fibers by scar tissue. The anterior tricuspid leaflet of this dog was infiltrated focally by neutrophils. The kidneys of both Dog 81 and Dog 97 showed a 3+ diffuse proliferative glomerulonephritis. There were a few intertubular collections of lymphocytes and plasma cells, but no focal glomerular lesions and no changes suggesting subacute or chronic glomerulonephritis were observed.

Fig. 8.—Organized vegetation on auricular surface of thickened anterior mitral leaflet of Dog 81, killed 55 days after an injection of Streptococcus mitis. Hematoxylin and eosin; × 15.

Effects of Cortisone.—The course of infection in the dogs given cortisone was similar to but usually milder than that in dogs given similar bacterial injections but no cortisone. The vegetations were generally smaller than in the noncortisone group, and none were noted in Dog 83 (Table), which received a single injection of S. mitis. Microscopically, the endocardial lesions and the inflammatory lesions in other organs in the dogs given cortisone showed no consistent striking differences from those seen in the dogs which did not receive cortisone. There were marked individual variations in the microscopic picture in both groups. All the dogs in the cortisone group showed increased cellularity of the glomeruli, which roughly paralleled the severity of the endocarditis (Table). None of the dogs given cortisone showed focal glomerular lesions resembling those described in Dog 66.

Comment

The present study shows that a single inoculation of bacterial culture is sufficient to produce endocarditis and a proliferative glomerulonephritis in dogs with aortic insufficiency. The course of the infection and the character and severity of the lesions are comparable to those produced by multiple injections of the same organisms.[1] This single-injection method presents several advantages. The picture is not complicated by multiple bacterial infections and reactions of variable duration and severity. This permits a more precise interpretation of the clinical course and pathologic changes at various stages of the disease. Accordingly, it will be helpful in various experimental procedures designed to study factors predisposing to the disease or affecting its course and in evaluating the efficacy of various forms of therapy.[7] An increase in cardiac work load has been considered a major factor in increasing susceptibility to experimental endocarditis.[1,6,8] We have used this single-injection method to show that insertion of a Hufnagel valve, which reduces cardiac work load, does not lessen the susceptibility of dogs with aortic

insufficiency to endocarditis and glomerulonephritis.[2]

In the present study, the natural course of infection with S. mitis was observed in four dogs. One (Dog 66 in Table) died in 12 days, because of rupture of a chordae tendineae, and one (Dog 105) died in 20 days with a severe infection. The other two were killed 55 and 203 days, respectively, after the bacterial inoculation and showed evidence of an arrested endocarditis. However, even though their blood cultures had become persistently negative beginning 44 and 114 days preceding death, both showed a 3+ diffuse proliferative glomerulonephritis. Neither showed changes suggesting subacute or chronic glomerulonephritis in man. This is of interest, since the concept of a relation between acute and chronic glomerulonephritis is being questioned.[9]

The major purpose of the cortisone study was to determine its effect on the endocardial and renal lesions. No significant consistent differences were noted in the microscopic appearance of the endocardial lesions. The severity of the lesions, which may be graded roughly by adding the plus signs in the three columns on the left in the Table, tended to be less in the dogs given cortisone. However, because the severity of the lesions varied markedly in different dogs given the same treatment, further studies are needed to evaluate the effect of cortisone on the severity of endocarditis.

The proliferative glomerulonephritis in our dogs resembled in many respects that produced by an antigen-antibody reaction. Rich and his associates[3] have stated that a single intravenous injection of sufficient protein to ensure residue in the circulation after the development of antibody causes glomerulonephritis. They have reported that cortisone prevents the development of such a diffuse proliferative glomerulonephritis in rabbits sensitized to horse serum. In our study, the administration of cortisone did not prevent the development of glomerulonephritis. The severity of the proliferative changes in the glomeruli tended to vary with the severity of the endocarditis rather than with the administration of cortisone. After injection of streptococci, the incidence of severe glomerulonephritis was lower in the dogs given cortisone, but the endocarditis in this group also tended to be less severe. After injection of staphylococci, the dogs which received cortisone and developed a severe endocarditis also developed a severe glomerulonephritis (Table). A glomerulonephritis developed in two dogs (Dogs 48 and 116) within three days after an injection of staphylococci; it seems unlikely that an antigen-antibody reaction could have produced such changes within this short period. It is probable, therefore, that factors other than an antigen-antibody reaction, perhaps bacterial toxins, play a major role in the genesis of the proliferative glomerulonephritis in this study.

Summary and Conclusions

A simplified method for the production of endocarditis, with use of dogs with aortic insufficiency, is described. An infection resembling acute and subacute bacterial endocarditis was produced consistently in 12 such dogs by injecting intravenously 1 cc. of a broth culture of Staphylococcus pyogenes var. aureus and Streptococcus mitis, respectively. Ten dogs that died or were killed within three weeks showed vegetations on the mitral or aortic valves or on the suture line of the aorta, visceral infarcts, and hemorrhagic and inflammatory lesions in various organs. Two dogs given streptococci and killed after 55 and 203 days showed an arrested endocarditis. All the dogs developed a diffuse proliferative glomerulonephritis which roughly paralleled the endocarditis in severity. Dogs treated with cortisone tended to have less severe gross endocardial lesions. Microscopic studies, however, revealed no consistent striking differences in the appearance of the endocardial and renal lesions between the dogs that did and did not receive cortisone.

National Institute of Arthritis and Metabolic Diseases (14).

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Histologic and Histogenetic Observations on the Peripheral Adenoma of the Lung

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The peripheral adenoma of the lung has always been an intriguing lesion, both diagnostically and pathologically. By peripheral adenoma is meant those lesions which cannot be visualized bronchoscopically. It is therefore obvious that the diagnosis is practically never made prior to operation.

From the pathologic standpoint all cases previously reported in the literature have been of the carcinoid type.[2,3,5,9] Most lesions have been solitary, but a few have been multiple,[2,10] and it is from these latter examples that a glimpse into the possible site of origin has been attained.

It is proposed in this paper, by illustrative cases, to elaborate further on this problem and particularly to bring into focus the possible relationship of the peripheral adenoma to the so-called benign epithelial proliferations which have been described by a number of investigators.[4,7,8]

Report of Cases *

CASE 1.†—A 59-year-old white woman was admitted to the hospital on June 2, 1953, complaining of cough and fever of four months' duration. Past history revealed a similar episode with pulmonary hemorrhage one year previously. A chest roentgenogram showed an oval density in the left upper lung field, 2 cm. in its greatest diameter, and located at the costochondral junction of the second rib. A left upper-lobe lobectomy was performed on the 10th hospital day, and the patient was discharged improved on June 27.

The patient was readmitted Sept. 5, 1953, because of persistent cough which was only mildly productive. There had been no hemoptysis since her first operation. Several chest roentgenograms showed small lesions in the right lower lung fields. The removal of the right upper and middle lobes was accomplished five days later, and the patient was discharged on Sept. 25, 1953.

The patient was admitted for the third time on April 20, 1954, with complaints of a dry, hacking cough. There had been no hemoptysis, fever, or loss of weight. A chest roentgenogram, prior to admission, had shown a density in the right lower lobe. The anterior and medial basal segments of the right lower lobe were resected on the third hospital day. The patient was discharged 11 days later to be followed at frequent intervals.

The final admission was on Sept. 18, 1956, when the patient suddenly developed dyspnea, substernal pain, and inability to lie down during the attack. These symptoms were attributed in part to a hiatus hernia, known to have been present since the patient's first thoracotomy. In addition there was hypertension and a slightly enlarged heart. A chest roentgenogram showed no further lung parenchymal lesions. The patient was discharged eight days later with instructions to take a low-salt diet and digitoxin.

Pathologic Findings.—First Operation, June 12, 1953: Gross description. The specimen consisted of the entire left upper lobe of the lung and a small amount of very friable lobular pale yellow tissue removed from this lobe for the purpose of frozen section study. The latter was approximately 3 cc. in volume. A frozen section of this material revealed what was thought to be a carcinoma of the oat-cell type. The main specimen measured 20 by 7 by 1.5 cm. and weighed 113 gm. It was soft, well aerated, and pink. The pleura was intact, and no nodules were felt or seen. Dissection of the bronchi revealed no gross neoplasm, but many prominent hilar lymph nodes were identified. These showed rather severe anthracosis but no evidence of tumor. Multiple parallel slices through the lung parenchyma showed an essentially normal structure.

Microscopic description. Sections through the biopsy specimen utilized for frozen section showed a cellular neoplasm composed of diffuse sheets of compactly arranged tumor

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From the Laboratory of Surgical Pathology, Medical College of Virginia.

* These cases will be presented in greater detail in a future paper now in preparation.

† Presented with permission of Dr. F. Philip Coleman.

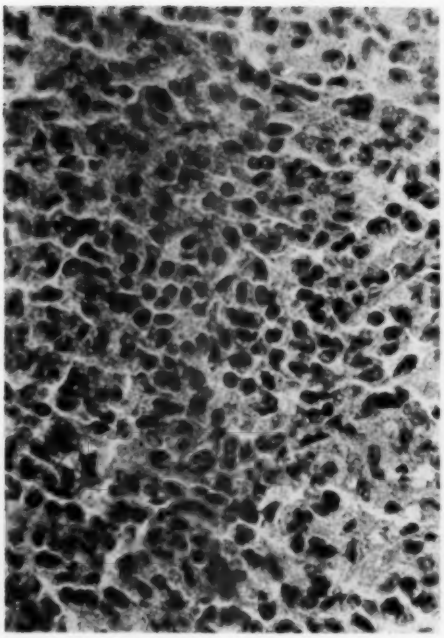


Fig. 1 (Case 1).—Solid mass of tumor cells from solitary neoplasm excised at biopsy from the left upper lobe. Note uniformity of the cellular architecture.

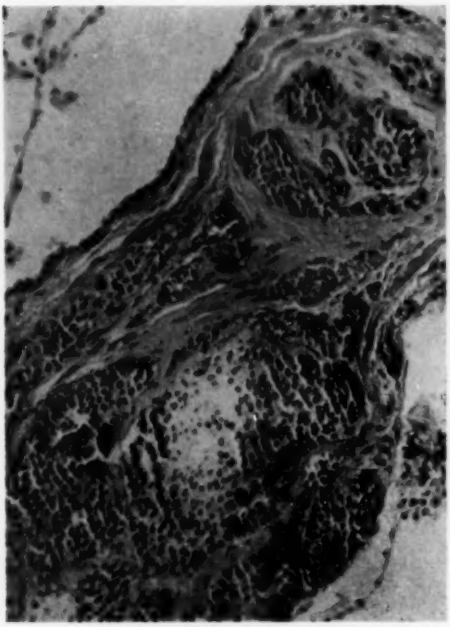
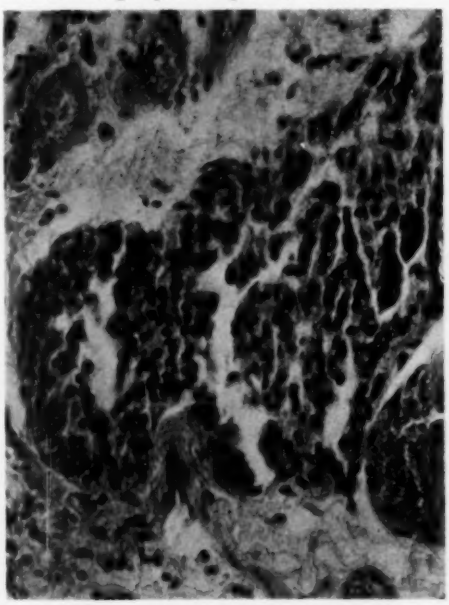


Fig. 2 (Case 1).—Groups of similar tumor cells in wall of bronchus adjacent to tumor mass on which biopsy was done.

cells with very little stroma (Fig. 1). The cells were arranged in no particular pattern, and no differentiating features were seen. The cytoplasmic borders were indistinct, and the cytoplasm was faintly eosinophilic and sometimes finely granular. The nuclei were uniform and generally round or ovoid. Mitoses were scarce. Sections through the biopsy site showed scattered foci of microscopic tumor. In many instances solidly packed groups of tumor cells were in intimate contact with the epithelium which lined a medium-sized and a small bronchus (Fig. 2). In other areas the tumor consisted of solid accumulations, varying from four to five cells to larger masses. These often showed no connection with bronchial walls (Fig. 3). These foci were also widely scattered, since they could be found in both the apex and the lingula of the lobe. A small focus was also found in the hilar region, but not connected with a bronchus. Examination of all lymph nodes showed no evidence of

Fig. 3 (Case 1).—Small groups of tumor cells remote from bronchial walls and resembling the so-called benign epithelial proliferations.



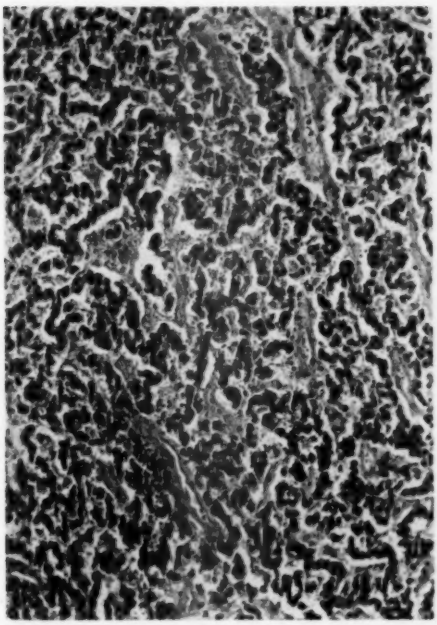


Fig. 4 (Case 1).—Microscopic features of one of the tumor nodules removed at the second operation. Note resemblance to carcinoid tumor.

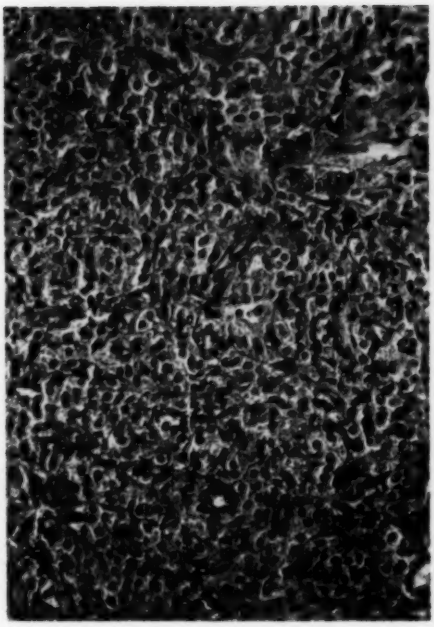


Fig. 5 (Case 1).—Another area of one of the tumor nodules, showing acinar groupings of neoplastic elements.

metastases. Silica granulomata and severe anthracosis was present.

Diagnosis: Peripheral adenomata of upper lobe of left lung.

Second Operation, Sept. 10, 1953: Gross description. The specimen of lung was submitted in four parts. The first was a segment of lung tissue from the right upper lobe, 4.5 by 3 by 1 cm. Two well-demarcated tumor nodules, 1.1 and 1.2 cm. in diameter, respectively, were identified. A frozen section of one of these nodules showed tumor entirely similar to that described at the first operation. The nodules were tan, lobulated, and firm. They were subpleural, and the overlying pleura was smooth and shiny. The surrounding lung parenchyma was pink, soft, and crepitant. The second specimen was also from the upper lobe and consisted of a small piece of tissue 2.4 by 0.5 by 0.4 cm. A small cystic structure, previously opened, 0.9 cm. in diameter was found. Its lining was thin and partly opaque. The third specimen was the middle lobe of the lung, 10 by 6.5 by 1.4 cm. and weighing 235 gm. The pleura was smooth and shiny except for a small ragged area due to surgical excision of a portion of lung tissue. The parenchyma was soft and crepitant, without gross evidence of neoplasm. Finally, there was a small piece of middle lobe 2.4 by 1 by 0.3 cm. No pathologic features were discerned.

Microscopic description. The tumor nodules consisted of sheets of small uniform cells with finely granular cytoplasm and round or oval nuclei. In some areas the cells were arranged in strands with interspersed stroma rich in blood vessels (Fig. 4). In other areas the tumor cells were arranged in rounded and irregular groupings which sometimes suggested acinar formations (Fig. 5). Multiple sections through the middle lobe revealed numerous foci of tumor, some of which appeared to arise from the basal layer of the bronchial epithelium (Figs. 6, 7, and 8). In the smaller collections the tumor cells were often spindle-shaped and palisaded (Figs. 9 and 10). A section through the cystic structure of the upper lobe showed an emphysematous bleb with a thick, hyalinized, collagenous wall. A nest of tumor was seen in the vicinity.

Diagnosis: Peripheral adenomata of upper and middle lobes of right lung.

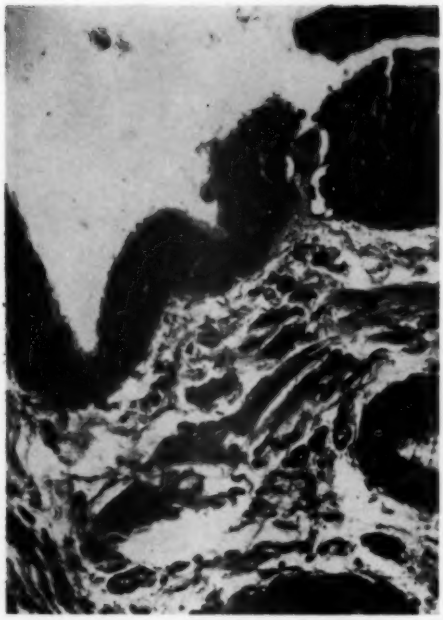


Fig. 6 (Case 1).—Ciliated bronchial epithelium with focus of epithelial proliferation in intimate contact with base of epithelium.

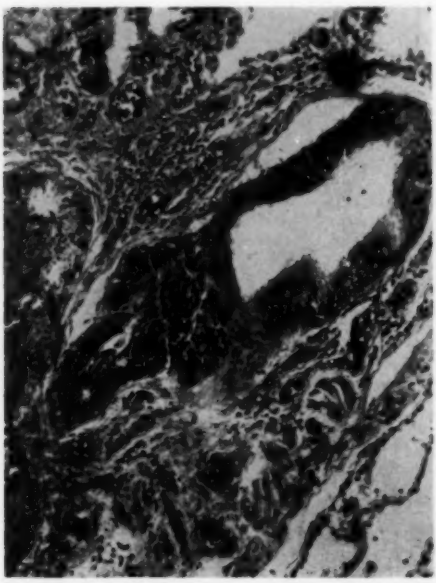


Fig. 7 (Case 1).—Medium-sized bronchiole with epithelial proliferation streaming away from bronchiolar wall into surrounding pulmonary parenchyma.

Fig. 8. (Case 1).—High-power view of Figure 7. Note apparent origin of the epithelial proliferations from the deep cells occupying the wall of the bronchus.

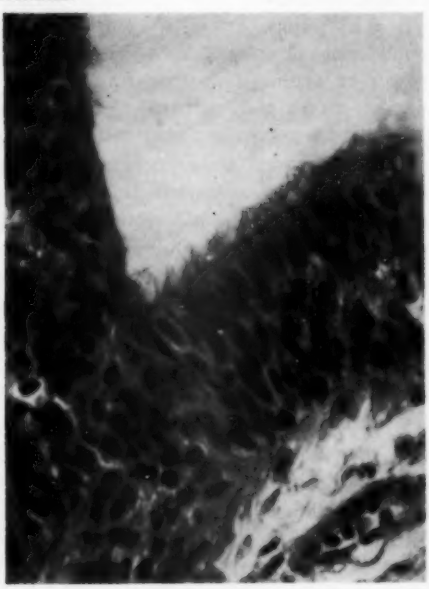


Fig. 9 (Case 1).—Terminal bronchiole surrounded by proliferating epithelial cells.

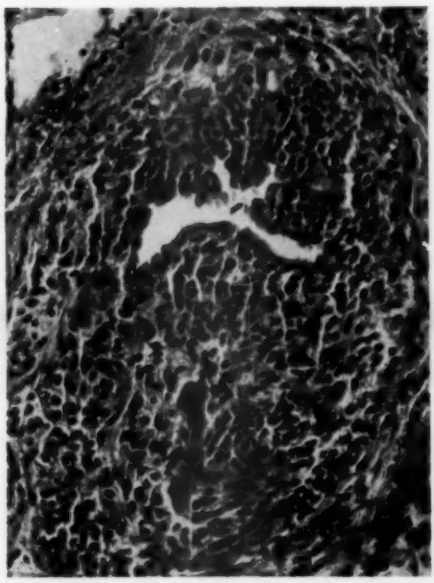


Fig. 10 (Case 1).—High-power view of Figure 9. Note spindle characteristics of some of the tumor cells.

Third Operation, April 22, 1954: Gross description. The specimen consisted of the anterior and medial basal segments of the right lower lobe, 9.5 by 6 by 1 cm. The pleura was in general smooth and shiny, with the exception of one surface, covered by fibrous adhesions. On cut section there was a protruding, firm tumor nodule 1.7 by 1.2 by 1 cm. (Fig. 11). The nodule was adjacent to a secondary bronchus, but origin from this bronchus or any other could not be demonstrated. The cut surface of the nodule was light gray-brown, with slight wrinkling. The surrounding lung parenchyma was unremarkable.

Microscopic description. The nodule was composed of uniform compactly arranged cells, entirely similar to the tumor cells described above. Compartmental groupings were often observed, as well as pseudoalveolar zigzag patterns. Occasional mitoses were found. Scattered throughout the lung parenchyma were small epithelial collections resembling the benign epithelial proliferations, sometimes noted in cases of bronchiectasis. These were found adjacent to bronchiolar walls or as isolated nests which appeared to be in alveolar spaces. Some of the nests were in intimate contact with the basal cells of terminal bronchioles.

Fig. 11 (Case 1).—Resected pulmonary segments at third operation to show circumscribed tumor nodule.

Diagnosis: Peripheral adenomata of anterior and medial basal segments of the right lower lobe.

Case 2.‡—A 34-year-old white woman was admitted to the hospital on May 23, 1957, complaining of left-sided chest pain present for approximately 10 years. This has recently been aggravated by motion, deep breathing, and coughing. A chest film taken before admission showed a 2 cm. coin lesion in the left midlung field. A thoracotomy was performed on the seventh hospital day. A wedge excision of the lesion was done, after which the wound was closed. Five days later the thoracotomy incision was reopened, and a total pneumonectomy was carried out. The patient was discharged home ambulatory on June 15, 1957, in good condition, to be followed as an outpatient.

Pathologic Findings.—Biopsy Specimen: Gross description. The specimen consisted of a piece of lung parenchyma from the lingular segment of the lung. It measured 3 by 2 by 0.3 cm., was moderately soft, and showed no nodules. A frozen section was interpreted as a granulomatous lesion, and no further surgery was done.

Microscopic description. Sections showed an epithelial neoplasm composed of diffuse sheets and variously sized nests of compactly arranged cells with indistinct cytoplasmic borders (Fig. 12). The nuclei were uniform, round, or ovoid. Considerable desmoplasia was associated with the tumor. In some areas the neoplastic elements had a spindle shape,

‡ Presented with permission of Dr. James Brooks.

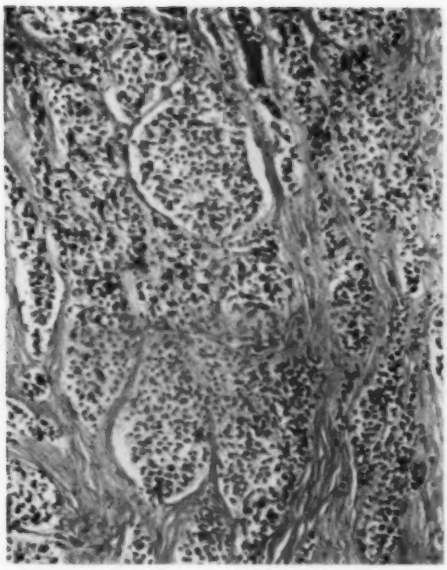


Fig. 12 (Case 2).—Nests of tumor cells in dense fibrous stroma. Note spindling of many of the tumor cells.

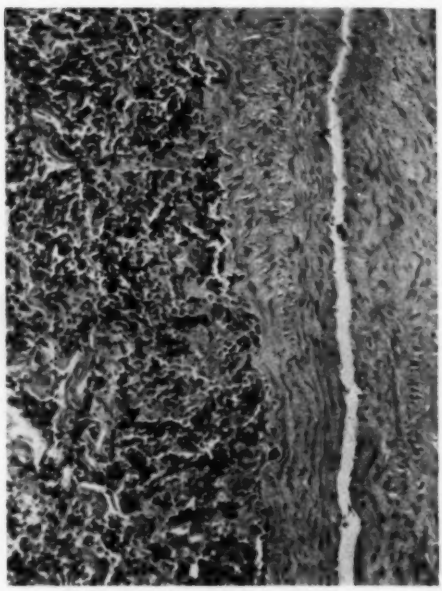


Fig. 13 (Case 2).—Tumor cells infiltrating adventitia of pulmonary artery.

with palisading of the nuclei. No differentiating features were seen, though there was a tendency of the epithelial tumor nests to be associated with clefts apparently representing alveolar spaces. Bronchial cartilages were invaded by tumor, but in no area could an origin from bronchial epithelium be discerned. A branch of the pulmonary artery showed invasion of the adventitia by tumor (Fig. 13).

Main Pulmonary Specimen: Gross description. The specimen consisted of the entire left lung, 21 by 15 by 4 cm. and weighing 354 gm. The pleura was smooth and glistening except over the apex and posterior aspect, where there was a fibrinopurulent exudate extending down to the diaphragmatic surface. The interlobar fissure was obliterated. On the medial surface, anterior to the hilus, was a sutured incision, 3 cm. in length. The lung surrounding this area was indurated. The remainder of the lung was soft and subcrepitant. In addition there was received pieces of parietal pleura and a number of bronchial and mediastinal lymph nodes.

Microscopic description. Multiple sections through the lung revealed small nests of tumor cells in the lingular segment adjacent to the area utilized for biopsy. These cells occurred in small groups and appeared to be largely confined to alveolar spaces. In addition, a section through the hilum showed a 1 mm. focus of tumor. This appeared to consist of a compact group of alveolar spaces filled with small uniform epithelial cells. These cells were entirely similar to those often described as benign epithelial proliferations of the lung. An adjacent bronchopulmonary lymph node contained small nests of tumor cells in the peripheral sinus. One bronchial node also showed partial replacement of the paranchyma by metastatic tumor (Fig. 14). The remaining lymph nodes (a total of 30 examined) were negative for metastases.

Diagnosis: Peripheral adenoma of lingular segment of left upper lobe of lung with metastases to bronchopulmonary and bronchial lymph nodes.

Comment

The first case is unique in that considerable amounts of lung tissue required removal for extirpation of the tumors, namely, the left upper lobe, the right upper and middle lobes, and the anterior and medial basal

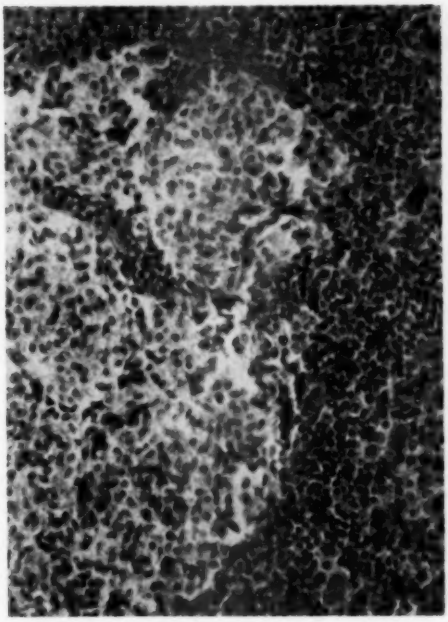


Fig. 14 (Case 2).—Bronchial node partially replaced by metastatic tumor.

segments of the right lower lobe. Not only were there definite tumor nodules in various segments of the lung parenchyma, but countless microscopic foci of epithelial proliferations were distributed throughout the pulmonary tissues. It was these foci that enabled one to study the early origins of these epithelial proliferations, particularly when they were related to small bronchi. In such areas one could clearly note the resemblance of the tumor cells to the reserve cell of the bronchiole, and it is not farfetched to envisage origin from this cell.

Further evaluation of these epithelial tumors required comparison to the so-called benign epithelial proliferations found in certain cases of pulmonary disease. A case of bronchiectasis studied in 1950 was useful for this purpose.§ In this case, scattered groups of epithelial accumulations were found in the periphery of the lung, where epithelized alveoli were a prominent feature (Fig. 15). In these areas the epithelial nests appeared to reside in spaces of various

§ This patient is known to be well at least five years after operation.

Fig. 15.—So-called benign epithelial proliferations in a case of bronchiectasis. Note "epithelized" alveoli in lower right margin.

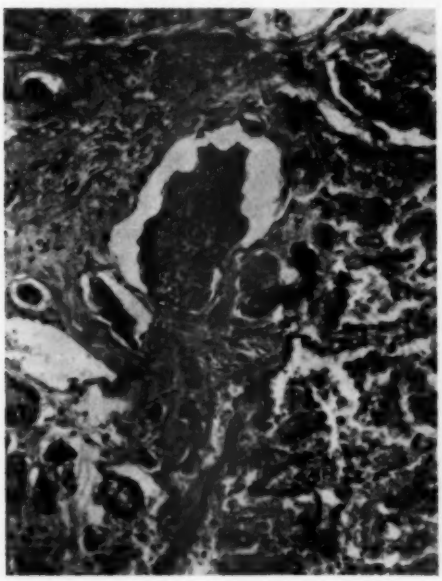
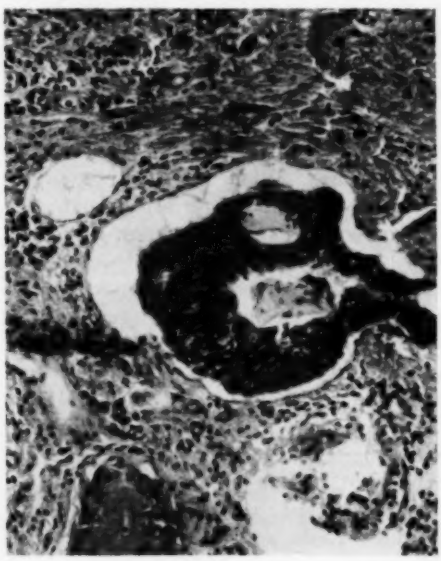


Fig. 16.—High-power view of one epithelial focus. The space surrounding the epithelial nest is thought to be artifactual.



sizes which simulated lymphatic spaces (Fig. 16). These spaces, however, were thought to represent artifacts due to shrinkage, and it was more logically concluded that they were terminal bronchioles and alveoli. The cellular component seems rather strikingly similar to many of the proliferations found in Case 1 (compare Figs. 2 and 15, 3 and 16). It is therefore not unlikely that whatever leads to the stimulation of these epithelial masses may ultimately lead to the formation of true tumors, either as a single mass or as multiple smaller nodules. This relationship between multiple peripheral epithelial proliferations and the carcinoid type of bronchial adenoma is not an original concept, since Prior [8] likewise thought the benign growths of his 12 new cases added to the literature were clinically and histologically similar to the adenoma in question.

The second case showed the unusual feature of metastasis to one bronchopulmonary and one bronchial lymph node. To the best of my knowledge metastases from peripheral adenomas of the lung have occurred in only one previously reported case. Good and Harrington [3] described 23 cases from the Mayo Clinic, and one of the patients, who did not survive operation, showed metastasis to the mediastinal lymph node and to the liver. An interesting by-product of this problem can be found in the paper of Prior and Jones.[7] These authors discuss the so-called benign epithelial proliferations in the lung and present six autopsy cases and two surgical cases. While the authors conclude that the proliferations are benign and without consequence if a long follow-up is possible, 1 case from 12 others found in the literature showed nests of tumor cells in the hilar node of the lung. The significance of this finding, however, needs to be evaluated. Certainly the usual hilar carcinoid type of pulmonary adenoma with regional lymph node metastases has not been incompatible with long-term cure. It is possible that the finding of a few epithelial nests of the "peripheral type" within lymph nodes only indicates facile accessibility to lymphatics but sluggishness in growth and lack of aggressiveness upon host tissues.

Summary

Two cases of multiple peripheral adenomas of the lung are presented. One case showed metastasis to regional nodes.

The presence of multiple cellular foci was helpful in studying the histogenetic possibilities, as well as the relationship of these foci to the so-called benign epithelial proliferations sometimes found in cases of bronchiectasis, interstitial fibrosis, and cystic disease of the lung.

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The Interaction of Cortisone and Vitamin D on Bones of Rachitic Rats

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A series of clinical papers presenting metabolic data suggestive of an antagonistic action between cortisone and vitamin D has been published recently.[1-6] The experiments to be described were designed to study the interrelationship between the actions of cortisone and vitamin D in rats. Since one of the most sensitive indices of vitamin D action is the healing of rickets, the design was to test the ability of cortisone to counteract this specific influence.

Material and Methods

Male albino rats weighing 45 to 50 gm. at the beginning of the experiment were used. As rats can absorb calcium from the intestine in the absence of vitamin D, rickets was produced by a low-calcium vitamin D-free diet (Table 1).

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From the Department of Pediatrics and Pathology, Harvard Medical School, and the Children's Medical Service and The James Homer Wright Pathology Laboratories.

TABLE 1.—*Composition of the Low-Calcium Vitamin D-Free Diet*

	Gm.
Na_2HPO_4	101.
NaCl	11.75
$MgSO_4 \cdot 7H_2O$	25.
Na I	0.075
$CuSO_4 \cdot 5H_2O$	0.1
$ZnCl_2$	0.022
$MnSO_4 \cdot 4H_2O$	0.8
$FeC_6H_5O_7 \cdot 3H_2O$	7.5
K Cl	35.75
$NaHCO_3$	45.5
Cystine	5.
Thiamine HCl	0.01
Riboflavin	0.04
Pyridoxin	0.01
Na pantothenate	0.08
Menadione	0.125
Nicotinic acid	0.250
Inositol	2.5
p-Aminobenzoic acid	5.
Casein	625.
Dextrose	1900.
Corn oil	125.
Tocopherol	0.3
Vitamin A	50,000. (I. U. units)
Choline dihydrogen citrate	20.

After 17 days of this regimen the animals were divided into nine groups, each composed of four animals, and the low-calcium diet was continued. Table 2 shows the treatment given to each group. At the end of a three-day period of treatment the rats were killed. The tibiae were dissected and fixed in 10% formalin. After decalcification in 5% HNO_3, the bones were embedded in paraffin, cut, and stained with hematoxylin and eosin.

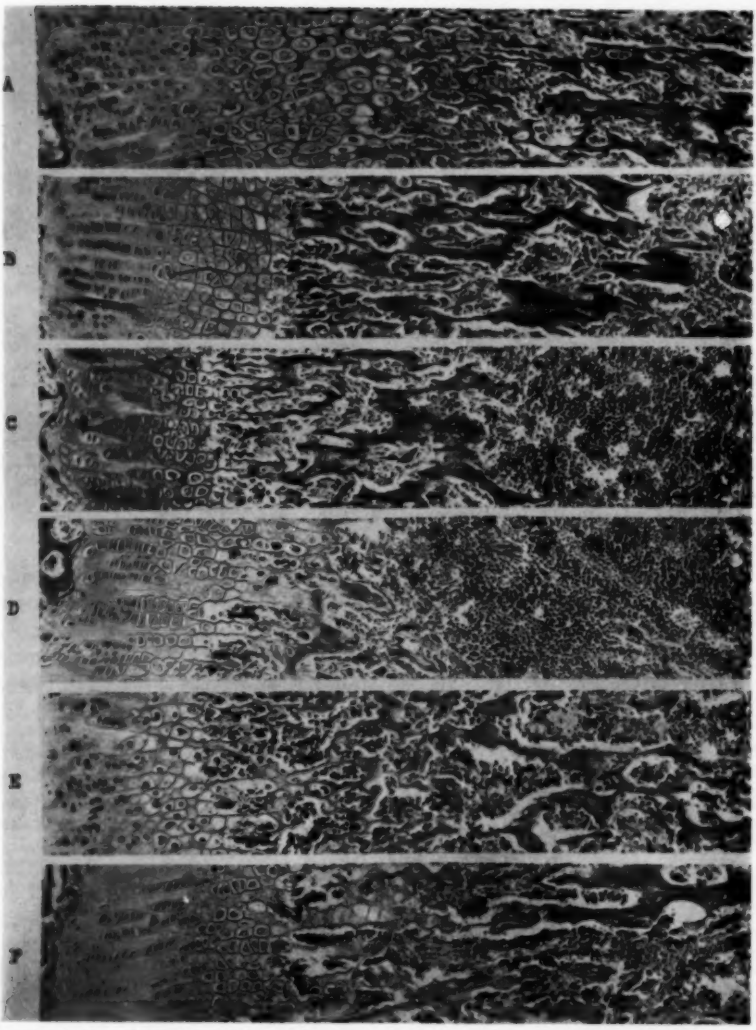
TABLE 2.—*Treatment Schedule of Rats from Seventeenth to Twenty-First Day of Low-Calcium Vitamin D-Free Diet*

Group No.	Daily Treatment *
1	Controls, no treatment
2	Cortisone 2 mg.
3	Cortisone 5 mg.
4	Vitamin D 100 I. U.
5	Calcium 80 mg.
6	Vitamin D 100 I. U. & calcium 80 mg.
7	Cortisone 5 mg. & vitamin D 100 I. U.
8	Cortisone 5 mg. & calcium 80 mg.
9	Cortisone 5 mg., vitamin D 100 I. U., & calcium 80 mg.

* Cortisone (Cortone Acetate, Merck Sharp & Dohme, Division of Merck & Co., Inc.) was injected intraperitoneally. Vitamin D and calcium were administered orally.

Results

Part *A* of the Figure shows the histology of the tibial epiphysis and metaphysis in a normal animal of the same age as those of the study group. Rats on the low-calcium vitamin D-free diet (Figure, *B*) show a diminution in the thickness of the epiphyseal cartilage at the expense of both the proliferative and the hypertrophic zones. The cells of the hypertrophic zone do not show the normal degree of swelling and vacuolation of the cytoplasm. The length and number of the newly formed trabeculae are diminished, and the width of the primary spongiosa also is decreased. The trabeculae forming the primary spongiosa are rimmed by osteoid



Longitudinal sections through rat tibiae; reduced 32% from mag. × 125. *A*, normal control. *B*, low-calcium rickets. Note thinning of epiphyseal cartilage and osteoid rimmed broad trabeculae. *C*, 2 mg. cortisone. *D*, 5 mg. cortisone given to rachitic rats. Note the marked thinning of the epiphyseal cartilage and disappearance of trabeculae. *E*, vitamin D. Healing, as mainly characterized by the length of trabeculae. *F*, vitamin D given concomitantly with 5 mg. of cortisone, showing combination of the changes seen in *C*, *D*, and *E*.

seams, which extend all the way to the diaphyseal surface. This is in contrast with the normal trabeculae, where the intercolumnar cartilage matrix persists for some distance before such broad osteoid seams are seen. The trabeculae in the rachitic bones are broad, plump, and club-like and composed of a thin central cartilage matrix with angular deeply basophilic contours. Osteoid seams are found in the shaft and the secondary spongiosa. With higher magnification one can see an occasional group of cartilage cells entrapped in the bony seams. The intercellular cartilage matrix spicule persists, thus lending the appearance of osteoid-filled cartilagenous lacunae. The vas-

cularity of the advancing bone and the number of osteoblasts between the newly formed bone are decreased.

As a result of treatment with 2 or 5 mg. of cortisone (Figure, *C* and *D*), there is a further decrease in the width of the epiphyseal cartilage, both proliferative and hypertrophic layers of cells being affected. A slight increase in cartilage bars with bony margins is discernible in both groups, this change being more evident in those treated with 5 mg. The length of the trabeculae is very much reduced, as is the width of the secondary spongiosa. Osteoid is present in discontinuous layers and is greatly reduced in comparison with non-cortisone-treated groups. As seen with higher magnification, there are fewer cuboidal osteoblasts. The appearance and number of the osteoclasts is not different from the untreated rachitic group. Similarly, there is no alteration in the intensity of the vascular supply.

Vitamin D and calcium when given alone or in combination alter the picture of rickets due to the low-calcium diet in a similar fashion (Figure, *E*). The epiphyseal cartilage remains thin. Here one sees an increased number of proliferative cells, and there are more binucleate cartilage cells than in the untreated rats. The hypertrophic cells appear to mature rapidly and to undergo rapid hydropic degeneration, as evidenced by their size, shape, and number. With high-power lens one sees that the lacunae left after necrosis of the hypertrophic cells are not invaded by capillaries but are filled with osteoblasts having small dark nuclei. There is a resurgence of growth as reflected by an increase in the width of the primary spongiosa. The newly formed trabeculae are thin and elongated and composed mainly of densely calcified cartilage matrix. The number and size of osteoblasts is increased. Osteoclasts appear reduced in number.

The effect of 5 mg. of cortisone given in addition to calcium or vitamin D is seen in the Figure, Part *F*. The epiphyseal cartilage is of essentially the same thickness as in the rachitic control group, but the hypertrophic cell layer is somewhat thinner. The rate of growth as indicated by the depth of the newly formed trabeculae appears to have been hindered. Nevertheless, signs of healing, increase in width of primary spongiosa as compared to the untreated rachitic rats, increase in the number and size of osteoblasts, and increase in vascularity persists. A finding not observed in any other group studied is the persistence of broad columns of cartilage matrix in the newly formed trabeculae. Similar changes have been found in normal rats treated with cortisone.[7] Rickets retards growth, and so the elongated cartilagenous columns necessary for this change are not formed.

Comment

In this investigation vitamin D in the presence of cortisone continued to induce healing, as shown by newly formed trabeculae and an increase in the number and size of osteoblasts. However, the extent to which these changes took place was reduced. Conversely, in the presence of vitamin D, cortisone continued to cause the characteristic picture of chondrolysis, with trapped cartilage cells in the developing trabeculae. Its antianabolic or catabolic effect was further evidenced by a reduction of osteoid.

The above findings constitute evidence that if vitamin D and cortisone are given simultaneously the characteristic action of one substance is not inhibited by the other. This may be considered as an argument that there does not exist an annihilating action or basic antagonism between vitamin D and cortisone as exists, for instance, between morphine and *N*-allylnormorphine. On the other hand, the ultimate result of their permissive interaction is that cortisone hinders the healing effect of vitamin D. Semantically this may be considered an antagonistic action, which resulted from separate actions on the same substrate of rachitic bone. In favor of this interpretation is the finding of independent actions of cortisone and vitamin D in teeth [8] and the recent observation that cortisone does not prevent the nephrocalcinosis induced by vitamin D in rats.[9]

Summary

Morphologic changes in the tibial epiphysis of rachitic rats induced by cortisone and vitamin D with or without calcium are described. The changes due to cortisone are very different from those resulting from vitamin D or calcium administration. When cortisone and vitamin D are given together the separate effects of each are evident; there is quantitative but no qualitative interference of the action of one drug with that of the other. The findings suggest that the two steroids, vitamin D and cortisone, have different sites of action.

Dr. H. R. Dudley gave criticism in this study.

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Subendocardial Hemorrhages of the Left Ventricle Following Trauma in Goats

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Subendocardial hemorrhages, predominantly of the left ventricle, have been observed for many years in a relatively small proportion of autopsies on humans. The literature indicates sporadic interest in the incidence, pathogenesis, and significance of these lesions.[1] Recent publications [2,3] stress the association, noted earlier by Berblinger [5] and others, of subendocardial hemorrhages with intracranial lesions and other anatomical changes that might lead to irritation of the vagus nerves. That direct stimulation of the vagi in dogs can evoke such hemorrhages was reported almost 50 years ago.[4,5]

In studying the pathology of wounding in experimental animals, the personnel of this laboratory have performed autopsies on many goats and have observed subendocardial hemorrhages in a considerable number of them. The findings in about 600 goats autopsied over a period of three years form the basis of this report.

Materials and Methods

The animals used were either grade goats procured in the eastern part of the United States or, more recently, white Angora goats from Texas. Each animal used in this study was wounded under controlled conditions and was allowed to survive until death occurred or, if it survived 48 hours, was killed at about that time, usually by electrocution. Almost all of the goats surviving for 48 hours appeared to be recovering successfully from their injuries and would no doubt have survived indefinitely in the absence of late complications, such as severe wound infections. During their periods of survival the animals were held in the laboratory and observed but usually were not treated in any way.

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From the Wound Assessment Branch, Biophysics Division, CWL.

The autopsies used in this report were performed between May, 1952, and September, 1955. Of the cases on record from this period, some had to be excluded because of deficiencies in the experimental or pathologic records and others were animals not subjected to wounding. All of the remainder, a total of 603, comprise the series under discussion. Of these, 89 cases were killed before the end of the standard 48-hour survival period and are excluded from the main series, leaving 514 in the latter.

Since subendocardial hemorrhages, when present, were almost invariably restricted to the endocardium of the left ventricle, only such cases are listed as positive or referred to as showing subendocardial hemorrhages below. In only a very few cases were there such hemorrhages in the right ventricle or the atria without any of the left ventricle, and rarely, in positive cases, there were also hemorrhages in the endocardium of other chambers of the heart. In the presence of wounds of the heart itself, hemorrhagic discoloration of the endocardium could often be seen immediately around the wound defect (for distances of 1 or 2 mm.). When this was the only hemorrhagic region, the rest of the left ventricular endocardium being free of discoloration, the case is listed as negative for subendocardial hemorrhages. No unusual difficulty arises in designating a case with a heart wound as positive or negative.

Sometimes there were present very striking and extensive subendocardial hemorrhages, and in other cases the hemorrhages, while not so large or numerous, still presented a perfectly clear-cut picture, permitting immediate gross diagnosis. Microscopic verification was not very difficult in such cases. In other instances, however, the gross picture was less definite, there being only a few vaguely outlined faint purplish regions. In some cases of this sort it has been noted that, after fixation and storage in 10% formalin, the endocardium no longer showed any discoloration at all and that microscopic examination did not reveal any hemorrhages. On the other hand, the obviously positive cases all remained so after formalin fixation. As a result of such observations, we have adopted the practice of making the final decision as to the presence or absence of subendocardial hemorrhage in every doubtful case on the basis of a reexamination of the fixed

Fig. 1.—Left ventricle laid open. Many subendocardial hemorrhages of small to moderate size distributed in characteristic fashion over projecting surfaces of papillary muscles and on interventricular septum. Spots on aorta are patches of melanosis. This goat died 12 hours after wounding.

gross material, supported by additional microscopic study. Even when microscopic reexamination was still negative, such grossly positive cases have been retained in the series as showing endocardial hemorrhages. Failure of microscopic verification occurred predominantly in animals dying within 5 minutes of wounding or surviving for 48 hours. In no case has subendocardial hemorrhage been found microscopically when there was no indication grossly of its existence in the fresh and fixed tissue.

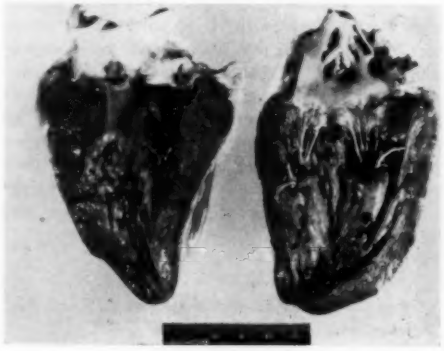
Description of Lesions

The gross and microscopic features of subendocardial hemorrhages, as seen in man, are well known, having been adequately described and illustrated in the literature. The picture in the goat is quite similar and requires no extended description.

The hemorrhages appear in the form of well-defined broad streaks or splotches of a bright red to dark purplish color, which stand out sharply against a pale pink or grayish background (Figs. 1 and 2). They vary in size and number from case to case. In the most marked instances dozens of individual hemorrhages ranging up to as much as 6 or 8 mm. in diameter are present. In other cases they are less numerous and smaller but otherwise retain the same characteristics. The hemorrhages are usually most numerous and largest over the projecting surfaces of the papillary muscles and on the interventricular septum. In many cases they are restricted to those regions, the portions of the left ventricular endocardium between the papillary muscles being free of hemorrhages. In the goat there is no apparent tendency for the hemorrhages to be more numerous over the larger branches of the conducting system than elsewhere. Purkinje fibers, which are very easily recognized under the microscope in this species, are found in abundance throughout the endocardium. Thus any subendocardial hemorrhages are certain to be near some elements of the conducting system.

A section through a typical hemorrhage shows numbers of extravascular red cells, usually most numerous in the subendocardium. (This is the name usually applied to the deepest layer of endocardium, in which the Purkinje fibers lie. The subendocardium is thus looked upon as part of the endocardium, rather than as a separate layer of tissue.) The hemorrhages often spread into the more superficial layers of endocardium and occasionally extend for very short distances into the interstitial tissue of the immediately underlying myocardium. The muscle cells beneath the hemorrhage ordinarily retain a normal microscopic appearance, but occasionally the cytoplasm is more eosinophilic than that in other parts of the myocardium, with some blurring of the finer cellular details. Frank necrosis of muscle

Fig. 2.—Left ventricle laid open. Many subendocardial hemorrhages, some very large. Largest hemorrhage covers tip of papillary muscle. The animal died 23 hours after wounding.



has not been found, however, except in a few cases in which minute foci of necrosis were present throughout the heart muscle. (Such focal necrosis of the myocardium is found occasionally in goats and is thought not to be related etiologically to the endocardial hemorrhages or to the experimental trauma.) Purkinje cells, even when surrounded by a hemorrhage, never showed degenerative changes.

Especially in cases showing grossly only a few small endocardial hemorrhages, microscopic demonstration of the lesions is not always easy. The difficulty is in part the obvious technical one of getting a section at just the right level. A further feature, noted frequently in hemorrhages of various sizes, is the fact that the extravasated red cells tend not to stain with their usual brilliance in hematoxylin-and-eosin preparations and can easily be overlooked during a cursory examination of the slide. These red cells usually retain distinct circular margins but have a washed-out appearance, as if they had lost much of their hemoglobin without being disrupted. Intravascular red cells in adjacent fields continue to stain normally. Recognition of this phenomenon resulted in the discovery of subendocardial hemorrhages on reexamination of the slides from several animals, in which they had not been found originally, even though it was known at the time that typical gross lesions were present. Such washed-out red cells are often more evident in preparations stained by the Masson or Gomori trichrome technique.

Results

Subendocardial hemorrhages occurred in 22% of 514 goats dying from wounds or killed after surviving wounding for 48 hours. These lesions are thus by no means uncommon as complications of trauma in this species. We are aware of no published estimates of their incidence after wounding or other trauma in man other than the data of Smith and Tomlinson discussed below, but it seems likely that they do not occur so frequently in man as in the goat.

TABLE 1.—*Association of Subendocardial Hemorrhages with Wounds of Various Organs*

Principal Organ Wounded	Subendocardial Hemorrhages, No. Cases		Total No. Cases	% with Subendocardial Hemorrhages
	Present	Absent		
Brain or spinal cord	10	41	51	19
Heart or major blood vessel	34	154	188	18
Lung	12	29	41	29
Liver	17	32	49	35
Gastrointestinal tract	10	47	57	18
Extensive soft-tissue damage	22	16	38	58
Other cases	10	80	90	11
Whole series	115	399	514	22

We have examined our cases in an effort to determine relationships between the location and severity of wounding and the incidence of subendocardial hemorrhages. The over-all incidence for wounds of various organs is shown in Table 1. When, as was often the case, several organs were wounded, the case was assigned to one organ only, in order to avoid overlapping in the entries, the precedence being assigned in the order in which the organs are listed in the Table.

It is apparent that there is considerable variation in the incidence of subendocardial hemorrhages among the different entries. Conclusions should not be drawn too hastily, however, since the differences reflect largely the variations in survival times. For example, about two-thirds of the cases listed under "heart or major blood vessels" died within five minutes after wounding, while only about 30% of the fatally wounded animals in this group survived beyond five minutes. In the cases of massive soft-tissue damage, on the other hand, only one goat died within five minutes, while 90% of those fatally wounded survived longer.

An examination of the incidence of subendocardial hemorrhages as related to survival time after injury yields information of more import. These data are displayed in Table 2.

In the column headed "survival time," the time intervals are to be understood to exclude the lower number and include the upper. Thus, a goat dying five minutes after wounding is listed under "0–5 min.,"

and not under "5–15 min." The shortest recorded survival time was 30 seconds. The presence or absence of subendocardial hemorrhages was determined according to the criteria mentioned above. The Table illustrates, incidentally, the pronounced tendency for goats subjected to wounding (predominantly by missiles) either to die promptly (i. e., within 1 hour after injury) or to survive for at least 48 hours. Only about 15% of the animals in this series suffered delayed death (i. e., death after 1 hour but in less than 48 hours). This distribution of survival times has been a striking feature in all of our results on goats used in experimental wound ballistics studies.

It is clear from Table 2 that subendocardial hemorrhages are found at autopsy only infrequently in goats surviving not more than five minutes. The shortest survival time associated with their presence was two minutes, there being 1 such case out of 21 animals dying so soon. Most (90%) goats dying within five minutes had wounds of the brain or cervical spinal cord, with extensive subarachnoid hemorrhage, or wounds of the heart or major blood vessels, with rapid massive loss of blood internally or, occasionally, externally.

At the other extreme, again with a low proportion of cases showing subendocardial hemorrhages, are animals surviving for 48 hours and killed. Among them are included many goats suffering only mild superficial wounds. Many of the animals having subendocardial hemorrhages in this group were rather severely wounded, sometimes as severely as goats dying of their wounds. Others had pneumonia (as did some not showing subendocardial hemorrhages). A few, however, showed no extensive or severe wounding and no serious disease of any kind.

As the Table indicates, the highest incidence of subendocardial hemorrhages was in goats dying between 15 minutes and 8 hours after wounding, with a still considerable incidence during the intervals just below and above those times. Indeed, while only about one-third of the goats died between 5 minutes and 24 hours, this set of animals included about two-thirds of the positive cases. Subendocardial hemorrhages were almost four times as common in this group as in the others.

TABLE 2.—*Incidence of Subendocardial Hemorrhages as Related to Survival Time*

Survival Time	Subendocardial Hemorrhages, No. Cases		Total No. Cases	% with Subendocardial Hemorrhages
	Present	Absent		
0– 5 min.	16	123	139	12
5–15 min.	21	45	66	32
15–30 min.	16	12	28	57
30–60 min.	8	4	12	67
1– 8 hr.	17	13	30	57
8–24 hr.	13	23	36	36
24–48 hr.	2	13	15	13
Surviving at 48 hr.	22	166	188	12
Whole series	115	399	514	22

The groupings of cases according to principal organs wounded have been examined with reference to survival times, and all show the highest incidence of subendocardial hemorrhages in the time interval just mentioned. A tabulation of this kind is presented for wounds of the brain and spinal cord (Table 3), since experience in man seems to indicate that the vast majority of cases of subendocardial hemorrhage are in persons suffering from intracranial lesions. The Table shows that the relatively low incidence of subendocardial hemorrhage in goats with wounds of the central nervous system is not to be accounted for by a too anomalous distribution of survival times. Most of the animals survived into the period of general highest incidence and failed to show even as many cases with hemorrhages as did animals not having brain wounds.

TABLE 3.—*Incidence of Subendocardial Hemorrhages in Cases with Wounds of the Central Nervous System*

Survival Time	Subendocardial Hemorrhages, No. Cases		Total No. Cases	% with Subendocardial Hemorrhages
	Present	Absent		
0– 5 min.	0	8	8	0
5–15 min.	6	12	18	33
15–30 min.	1	0	1	100
30–60 min.	0	2	2	0
1–48 hr.	0	4	4	0
Surviving at 48 hr.	3	15	18	17
All cases	10	41	51	19

One other group of cases is of sufficient interest to warrant separate tabulation (Table 4). Extensive soft-tissue wounds of the thighs and buttocks, often with fracturing of the femora, were inflicted on these animals. In many cases hemostasis and replacement of blood lost were carried out. Most of these animals were used for detailed physiological studies of wound shock and were subjected to preexperimental procedures, such as tracheotomy and catheterization of the vascular system, usually through the cervical vessels, often many hours before wounding. Such procedures and the concomitant restraint of the animal are, of course, themselves traumatic, and it is not too surprising that some of these goats died before the wounds were inflicted. Half of eight such unwounded cases showed subendocardial hemorrhages at autopsy.

The 91 goats that, for one reason or another, were killed before the expiration of the standard 48-hour period showed a lower incidence of subendocardial hemorrhage than those in the main series, but the highest incidence was in animals killed between 5 minutes and 8 hours. A good many of these animals were not too severely wounded and no doubt would have survived for 48 hours without showing subendocardial hemorrhages at any time.

The incidence of subendocardial hemorrhages was almost the same in males, females, and wethers, indicating that sex is not a determining factor in the development of the lesions.

In all cases care was taken to recover and measure free blood present in the serous cavities and in the form of hematomata in soft tissues. The cases from the main series were divided into those showing measured hemorrhage of 650 cc. or less and those showing more than 650 cc. (This amount of blood represents about 1.5 cc. per 100 gm. of body weight and is roughly the smallest amount of hemorrhage which can by itself be held to account for the death of a goat of average size.) The data from each group were examined, and in both it was found that the same preponderance of cases of subendocardial hemorrhage occurred in animals dying between five minutes and eight hours. The main difference between the two groups was a somewhat higher incidence of the lesions in cases surviving more than 30 minutes in the group with the larger blood loss. Massive blood loss, however, is clearly not essential for the development of subendocardial hemorrhages.

Cases having hemopericardium of 100 cc. or more (always associated with direct wounding of the heart or intrapericardial segments of the great vessels) showed an unexpectedly low incidence (14%) of subendocardial hemorrhages. A large proportion of these cases, to be sure, died within five minutes, usually from concomitant massive hemorrhage into the pleural cavities. However, of 18 dying between five minutes and eight hours, only 5 (28%) had subendocardial hemorrhages.

TABLE 4.—*Incidence of Subendocardial Hemorrhages in Cases with Extensive Soft-Tissue Wounds*

Survival Time	Subendocardial Hemorrhages, No. Cases		Total No. Cases	% with Subendocardial Hemorrhages
	Present	Absent		
0–5 min.	1	0	1	100
5–15 min.	1	2	3	33
15–30 min.	5	0	5	100
30–60 min.	1	1	2	50
1–48 hr.	12	12	24	50
Surviving at 48 hr.	2	1	3	67
All cases	22	16	38	58

Comment

The data just presented indicate that goats subjected to wounding by missiles or suffering extensive soft-tissue damage show subendocardial hemorrhages at autopsy very frequently, although by no means invariably. Of the 326 animals dying of their wounds within 48 hours, 29% had such lesions. The lesions are not associated predominantly with any particular type of wound or with wounds of any special organ or anatomical region. Rather, they appear to be related to the presence of nonspecific severe injury. It has not been possible to predict accurately in any given animal whether or not subendo-

TABLE 5.—*Comparison of Incidence During and After Time Intervals*

Time Interval	Fatally Wounded Animals Having Subendocardial Hemorrhages	
	Died During Interval, %	Survived Beyond Interval, %
0- 5 min.	12	41
5–15 min.	32	46
15–30 min.	57	43
30–60 min.	67	40
1- 8 hr.	57	29
8–24 hr.	36	13
24–48 hr.	13	--

cardial hemorrhages will be found at autopsy; almost any case showing them can be matched by another with the same type of wound and the same survival time in which the lesions are not present.

A conjecture regarding the time after injury at which the hemorrhages make their appearance in the endocardium is possible. Seldom do they occur in less than five minutes and never, in our experience, in less than two minutes. The figures in Table 5, which is derived from Table 2, suggest that the lesions usually arise within 30 minutes. This conclusion is based upon the unverified but fairly reasonable assumption that at a given instant the number of subendocardial hemorrhages in the surviving animals is not greater than that in animals dying in the immediately preceding period. On this basis, for example, not more than 32% of the goats surviving more than 15 minutes had subendocardial hemorrhages at that time, whereas 46% of them showed them ultimately, so that some must have acquired them after 15 minutes. On the other hand, not more than 57% of goats surviving beyond 30 minutes would be expected to have the lesions at that time, while actually only 43% did show them ultimately. This suggests that those actually found in such survivors were already present at 30 minutes. It will be noted in Table 5 that after 30 minutes the figure in the right-hand column remains less than that in the left, and so the effect just pointed out is at least consistent.

That the appearance of subendocardial hemorrhages is, at any rate, not always an agonal event is shown by the findings on animals killed, usually while still in good condition and not moribund, in less than the standard 48 hours. Of these goats, 15% had subendocardial hemorrhages, the lesions occurring as early as four minutes after wounding. The distribution with respect to time when killed was very similar to that of goats dying of their wounds. In our judgment, the ages of the lesions, which in no case could have been much more than 48 hours, could not be estimated with any degree of assurance from their gross or microscopic features. We have never, in this series of goats or in others, recognized with any degree of certainty resolved or healed endocardial hemorrhages, nor have we found hemosiderin deposition in any of them.

The pathogenesis of the subendocardial hemorrhages is obscure. We shall not discuss or try to augment the speculations that have been advanced. Any adequate explanation must account for the fact that the lesions are practically always confined to the left ventricle and for their characteristic distribution, as already described, in that ventricle.

Our results scarcely suggest that direct vagal irritation is of importance in most cases in the goat. It has already been pointed out that in our animals subendocardial hemorrhages were not unusually frequent after wounds of the central nervous system as compared with other wounds. Fassbender and Wengler [2] report 32 cases of endocardial hemorrhages in man, in all of which there were pathologic anatomical changes that would be expected to have produced vagus stimulation. In most of their cases there were intracranial lesions causing increased intracranial pressure or edema of the brain. In others, changes were present in the neck or mediastinum that might well have led to vagus irritation peripherally. These authors do not state what proportion of all cases with such anatomical changes showed subendocardial hemorrhages at autopsy, nor do they say how frequently, if at all, such hemorrhages were found in other kinds of cases.

Smith and Tomlinson[3] found subendocardial hemorrhages in 29 out of 235 human patients with fatal intracranial disease, an incidence of 12%. Of these 29 cases, 11 were instances of head trauma. Subendocardial hemorrhages were found in 20% of their autopsied cases of head trauma but in only 10% of other cases with intracranial lesions. In 607 autopsies on patients without fatal intracranial disease they found only 3 cases of subendocardial hemorrhage, all in persons not the victims of mechanical trauma. Their opinion is that "some hemorrhages must occur at the time of the sudden upset of intracranial conditions, rather than terminally."

As already mentioned, some of our goats showed endocardial hemorrhages after operative procedures in the cervical region, even though they were not otherwise subjected to trauma. This has also been noted in some, but by no means all, other goats subjected to similar manipulations, not included in the present series. In the few cases of goats in our series wounded in the neck and showing much hemorrhage and diffuse edema of the interstitial tissues, no endocardial hemorrhages were present, even though the animals survived well into the periods of highest general incidence. Such cases appear to resemble anatomically some reported by Fassbender and Wengler.

Nieberle[6] remarks that subendocardial hemorrhages are frequent in kosher-slaughtered (*geschächteten*) animals and relates their occurrence to the persistent beating of the empty heart after the rapid exsanguination (*Leerpumpen des Herzens*). He thus favors a purely mechanical etiology of the lesions.

Data on man at all comparable to those here presented on goats are not available, but it seems likely that if endocardial hemorrhages occurred in man after gunshot wounds or other trauma with anything like the frequency that they do in goats the fact would be well known and adequately documented. No doubt there is a real difference between the two species in this respect.

Summary

Subendocardial hemorrhages of the left ventricle were found at autopsy in 22% of 514 goats subjected to experimental wounding. Of the 326 animals dying from their wounds within 48 hours, 29% showed such hemorrhages.

The hemorrhages were found rather infrequently in goats dying in less than 5 minutes or killed after surviving 48 hours. They were commonest in animals surviving more than 5 minutes but dying within 24 hours of injury, occurring in as many as two-thirds of the cases dying between 30 and 60 minutes after wounding.

The frequency of subendocardial hemorrhages did not vary appreciably among goats with wounds of various anatomical regions or organs, being, in particular, no commoner after wounding of the brain or spinal cord than after wounding of other organs. The hemorrhages appear to occur as a nonspecific response to severe injury.

The data presented suggest that the subendocardial hemorrhages probably occur within 30 minutes of injury in most cases. Anatomical findings that tend to implicate direct stimulation of the vagus nerves in the pathogenesis of the subendocardial hemorrhages, such as have been described in almost all recently reported human cases, were encountered in only a small proportion of the goats.

Most of the goats were obtained from experimental work carried out by or under the direction of Dr. A. J. Dziemian. Many of the autopsies were performed by Dr. M. L. Washburn.

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Cytochemical Effects of Azaserine and DON on Ribonucleic Acid of Ehrlich Ascites Tumor Cells

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In earlier reports[1,2] it was noted that when Ehrlich ascites tumor cells were treated with azaserine (O-diazoacetyl-L-serine), the concentration of deoxyribose nucleic acid (DNA) decreased while nuclear size increased, with no alteration in DNA class distribution.

In these same cells, total protein increased, however, with increasing size. In the present investigation the behavior of ribonucleic acid (RNA) in the Ehrlich tumor cells in response to treatment with azaserine and DON (6-diazo-5-oxo-L-norleucine) was studied. This was of particular interest because RNA synthesis supposedly is directly proportional to protein synthesis if we accept Caspersson's theory on the mechanism of protein formation.[3] Recent work of Ben-Ishai and Volcani[4] has given support to this theory. Preliminary study of the action of azaserine hinted that there could be an exception to this.

Methods

Mice given injections intraperitoneally of Ehrlich ascites tumor cells were divided into three groups. One group was given injections intraperitoneally each day, starting 24 hours after tumor inoculation, for six days with 10μg. of azaserine per mouse (approximately 0.4 mg. per kilogram). Another group was given injections in the same manner of 0.2μg. of DON (a drug whose action is similar to that of azaserine) per mouse, and a control group was given injections of saline. At the end of the treatment period, ascitic fluid was withdrawn from each mouse, and smears were made and fixed while wet with Carnoy's fluid. These smears were stained with azure B (0.25 mg. per milliliter at pH 4 for two hours at 37 C) for histochemical evaluation of RNA as described by Flax and Himes.[5] Slides of treated and control cells were stained together.

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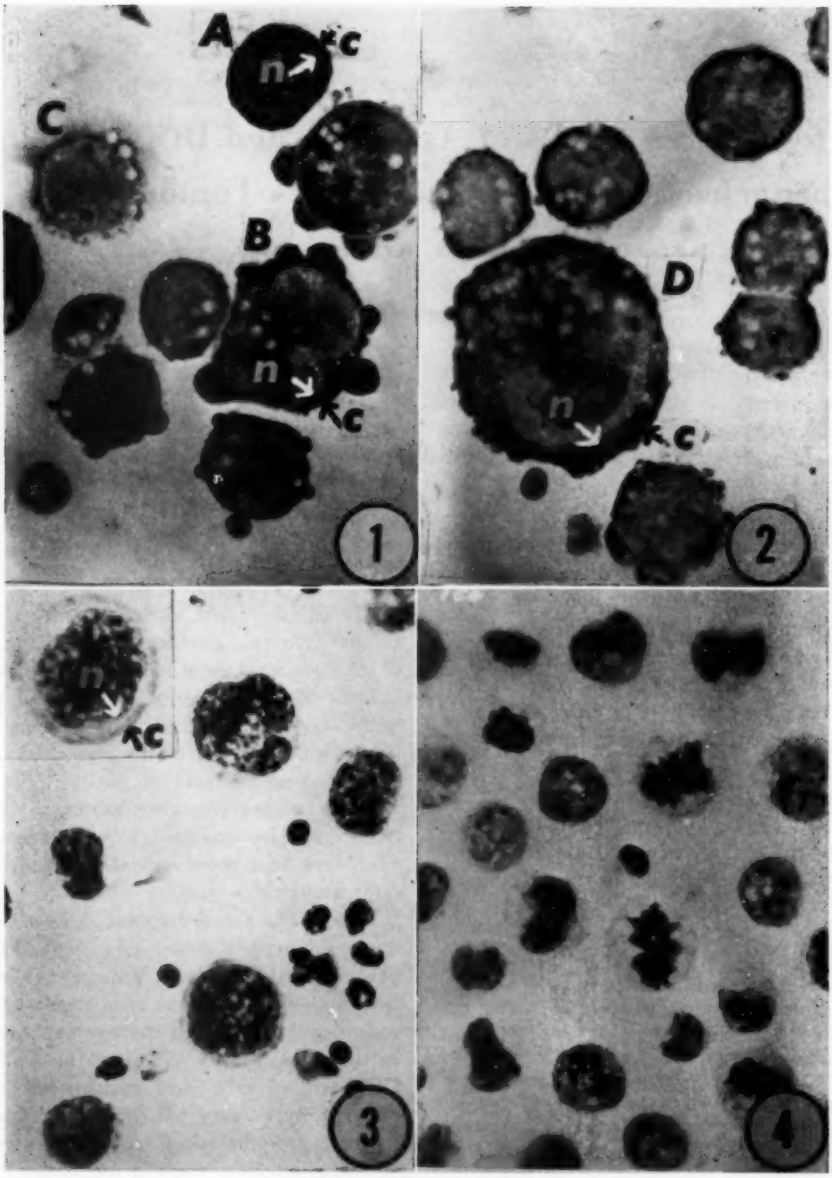
Qualitative Histochemical Evaluation

The RNA of the azaserine- and DON-treated cells was evaluated by the intensity of the stain as seen with monochromatic light of 600 mμ wavelength. With this stain under these conditions a dark-blue-staining cytoplasmic rim which was presumed to be RNA was seen around the nucleus (Fig. 1). In some of the enlarging azaserine-treated cells, dark-blue-staining globular structures (bubbles) were noted extruding through the cell membrane.

Besides these dark bubbles, other changes due to the drug treatment were marked cytoplasmic vacuolation and a decreased intensity of the blue stain as compared with the control cells. This cell damage appeared to be the same in both the azaserine- and the DON-treated cells and corresponds morphologically to early cell death. To identify the nature of the substance which extruded in these bubbles, enzyme extraction with ribonuclease was carried out. This was done with use of a solution of 1 mg. per milliliter of ribonuclease for two hours at room temperature. After this enzyme treatment, the globular structures were no longer seen, and all that remained were faint shadows outlining the cell membrane (Figs. 3 and 4). It was thus assumed that these bubbles contained RNA.



Qualitative Histochemistry

Fig. 1.—Treated Ehrlich ascites tumor cells stained with azure B. Cell *A* shows black rim containing RNA between nuclear membrane (*n*) and cell wall (*c*). Cell *B* shows enlarged nucleus with intact nuclear membrane; black globules containing RNA extrude from cell wall. In Cell *C* the cell wall has disintegrated; cytoplasmic vacuoles surround nuclear membrane; × 1000.

Fig. 2.—Treated Ehrlich ascites tumor cells. Cell *D* is similar to *B* in Figure 1 but is further enlarged, with beginning disintegration of nuclear membrane, as well as cytoplasmic vacuolation and nucleolar enlargement; × 1000.

Fig. 3.—Treated Ehrlich ascites tumor cells stained with azure B and after ribonuclease extraction of RNA. The black rim between nuclear membrane and cell wall has disappeared, as have the black globules; × 1000.

Fig. 4.—Untreated control tumor cells after azure B staining and ribonuclease extraction, similar to Figure 3 except for the larger number of smaller cells with five mitoses; × 1000.

The most interesting observation is in the comparison of the nuclear membrane with that of the cell wall. While the nuclear membrane apparently is capable of marked extension and expansion without signs of DNA extrusion, the cell wall seems to permit the RNA to escape readily and disintegrates before the signs of nuclear membrane damage are observed (Figs. 1 and 2).

Quantitative Histochemical Evaluation

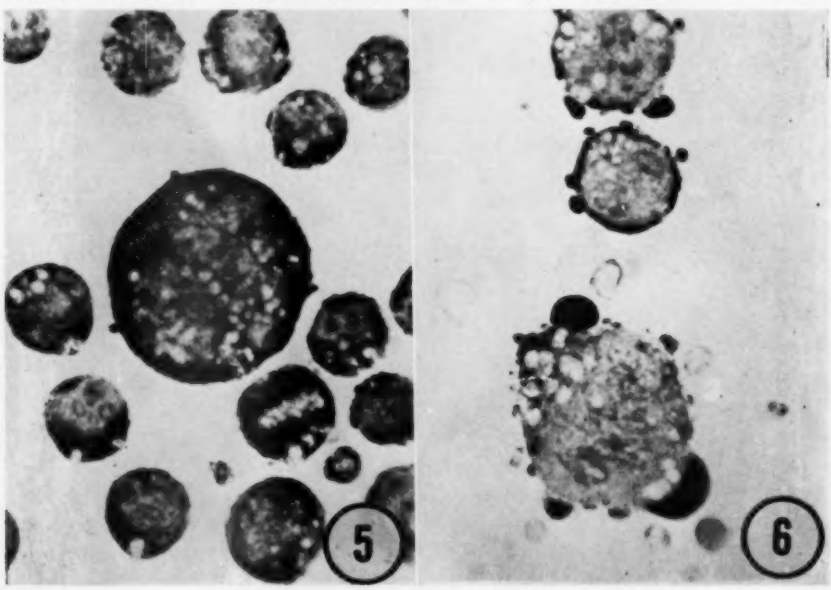
For quantitative evaluation of relative amounts of RNA, Flax and Himes [5] have shown that Beer's law can be assumed to be valid. Azure B stain was used in a concentration of 0.25 mg. per milliliter at pH 4, and the light absorption was measured with 600 mμ wavelength light. Randomly selected azure B-stained cells of azaserine- and DON-treated and control mice of replicate experiments were used. As the azure B stains both the DNA and the RNA, the DNA was extracted with deoxyribonuclease (DNAse) used in a concentration of 1 mg. per milliliter. The activity of the DNAse was checked with use of Kurnick's method of binding of methyl green.[6] The fading of the green stain of nuclei in the area of DNAse application demonstrated that there had been satisfactory activity of the enzyme. From this it was concluded that all the blue-staining substance after the DNAse treatment was RNA only.

Light absorption was evaluated by means of photographic photometry.[3,7] For the photomicroscopy Kodak's Verichrome Pan Film [8] was used and illumination was provided by monochromatic 600 mμ light. Exposure times were chosen to give density measurements on the straight-line portion of the characteristic curve of this film. A calibrated step wedge was used for emulsion calibration. Processing was carefully controlled (temperature, agitation, and time of development) and was carried out to

Quantitative Histochemistry

Fig. 5.—Azure B-stained control tumor cells after DNA extraction with deoxyribonuclease. Five times as many cells are seen as compared with treated cells in Figure 6; × 1000.

Fig. 6.—Azure B-stained treated tumor cells after DNA extraction. Large extruding bubbles are seen outside the cell membrane; vacuolation is also seen. These treated cells show decreased density as compared with control cells in Figure 5; × 1000.



RNA Inhibition by DON

Tumor Cells, No.		RNA Total Relative Am't. (Dens. Diff. × Area)	RNA %
Control	30	4,260	100
Treated	30	2,266	53

obtain a gamma of 0.7. It was assumed that the cells were flattened disks, and, therefore, the relative amounts of RNA were obtained by multiplying the area by the density differences[9] as obtained with a transmission densitometer.

It was found that when the cells from the control animals (Fig. 5) were compared with those of the treated animals (Fig. 6), the treated cells showed a decrease in relative amounts of RNA per cell of about 53% when the total amount contained in cytoplasm and nucleus was measured. This would seem to indicate RNA synthesis inhibition (Table). This was similar to the findings of LePage and associates,[1,10-13] in studies of the effects of azaserine on the in vitro incorporation of glycine-2-C^{14} into Ehrlich ascites tumor cells, in which it was found that the *de novo* purine synthesis was inhibited essentially completely. The findings in the present study suggest that the RNA synthesis is inhibited in treatment with azaserine and DON while protein synthesis proceeds. It seems that in such cases there is no direct relationship between the synthesis of the two. A possible explanation for this might be that, although the drugs block the *de novo* synthesis of purines, preformed purines are still available for the formation of RNA, which then can be used for protein synthesis.

It might also be of interest to mention the possible mechanism of action in the binding of the dye azure B with RNA when Ehrlich ascites tumor cells are subject to azaserine treatment. With such treatment some decrease in dye binding might be due to depolymerization of the nucleic acid. Since protein synthesis appears to continue while nucleic acid synthesis is arrested, we certainly could expect interference and decrease of dye binding due to this relative protein increase.[14]

Conclusions

Treatment of Ehrlich ascites tumor cells with azaserine and DON led to diminished binding of the dye azure B in the presence of an increase in the protein content of these cells. This, taken together with the evidence of LePage and his associates and the observation of extrusion of RNA-containing blebs, suggests a diminution of RNA in the cells and possible interference with the synthesis of RNA.

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Kimmelstiel-Wilson Glomerulonephropathy

Its Occurrence in Diseases Other than Diabetes Mellitus

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In their original description, Kimmelstiel and Wilson [1] implied that there was no fundamentally new or specific process involved in the genesis of diabetic glomerulosclerosis, which they equated with an exaggeration of the degenerative processes commonly seen in senile axial sclerosis. Newburger and Peters,[2] in 170 assorted control cases, were unable to find Kimmelstiel-Wilson lesions in any save in the case of a boy of 14 years of age dying of subacute glomerulonephritis, and in this case there were only a few suggestive lesions. In a study of the kidneys of 105 consecutive diabetic cases, 100 consecutive nondiabetic hypertensives, 100 normotensive nondiabetics, and 34 cases of Bright's disease, Allen [3] found typical lesions in three hypertensive cases in which the diagnosis of diabetes could not be made or excluded with certainty. In the 34 cases of glomerulonephritis, Allen [3] found 5 in which there were lesions suggestive of, or resembling, diabetic change. Siegal and Allen [4] recorded 1 instance of typical Grade 3 nephropathy in 100 consecutive cases of nondiabetic hypertensives, and in this case hyperglycemia and glycosuria were excluded. These authors also mentioned the accidental finding of one other instance of the occurrence of advanced lesions in nondiabetic nephrosclerosis and concluded with the opinion that "The theory that diabetes is the sole etiologic factor in the development of this form of glomerulosclerosis is thus rendered untenable."

Herbut,[5] in a review of 2000 autopsies, found 9 positive diabetic cases and 1 case in which the clinical data were inconclusive and regarded the lesions as specific for diabetes. Bell [6] emphasized that practically the same lesion may be found in glomerulonephritis, while Horn and Smetana [7] claimed to have found "intercapillary" glomerulosclerosis in 32 of 126 cases of arteriolar nephrosclerosis without diabetes, in 13 of 199 cases of glomerulonephritis, and in 1 case of cirrhosis with the Banti syndrome. Henderson and co-workers [8] found Kimmelstiel-Wilson type lesions in the kidneys nephritis and in 5.2% of 134 cases of hypertension. Rogers and Robbins,[9] while mentioning the finding of occasional instances of this type of glomerulonephropathy in apparently nondiabetic cases at the Mallory Institute of Pathology, stated further: "In each such case a painstaking search into the past history has uncovered the existence of diabetes mellitus at some earlier date." Thus, despite the occasional occurrence of similar renal lesions in other diseases, most authors agree that the severer type of nephropathy is always, or almost always, associated with diabetes mellitus.[7,8]

Materials and Methods

In the hope that further light might be shed on the nature, genesis, and etiology of the Kimmelstiel-Wilson lesions, we analyzed material available to us from 767 consecutive autopsies. Routine hematoxylin-and-eosin-stained sections of kidneys from these cases were examined for the presence of lesions resembling those of true Kimmelstiel-Wilson nephropathy. This survey revealed that lesions of this type were found in association with five types of disease entities, namely, necrotizing pancreatitis with fat necrosis, alcoholic fatty livers, hypertension, chronic glomerulonephritis, and traumatic fat embolism where the patients survived for a few days after trauma.

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From the Department of Pathology, the General Hospital.

Further examination in these cases included frozen sections of lung and kidney and in many cases of brain stained by oil red O and Mayer's hemalum. In addition, frozen sections from kidneys and hearts of 250 consecutive autopsy cases were stained and examined for lipids by the oil red O technique. In cases where Kimmelstiel-Wilson-like lesions were sufficiently abundant they were studied by the benzidine and Nadi staining techniques applied to frozen sections.

Results

Of 20 cases showing evidence of either diffuse or focal necrotizing pancreatitis accompanied by fat necrosis. Kimmelstiel-Wilson type lesions were found in the kidneys of 12. In three of these, the lesions were very scanty, but in nine they were sufficiently abundant to indicate or suggest some causal relationship (Fig. 1).

In our series of 33 cases of alcoholic fatty infiltration of the liver, 6 showed lesions of the Kimmelstiel-Wilson type in the kidneys, while of 23 cases of alcoholic portal cirrhosis, 4 had early or slight lesions of this nature. These categories were then studied for evidence of fat embolization, but in the case of alcoholic fatty and cirrhotic livers this investigation was confined largely to those showing Grade 2 or more fatty infiltration of the liver. Formalin-fixed blocks of lungs and kidneys were selected so as to give a size as near as possible to 3×2 cm. Frozen sections of these were cut—lung at 25μ and kidney at 15μ. The number of fat emboli seen in each section was counted. In the 20 cases of focal or diffuse pancreatitis, 11 showed fat embolization. Of 24 cases of fatty liver investigated in this way, fat emboli were found in the lungs of 13, and fat emboli were demonstrable in the lungs of 6 of 11 cases of alcoholic cirrhosis in which fat was relatively abundant in the livers.

In 46 cases of benign hypertension, 4 cases showed lesions resembling the Kimmelstiel-Wilson type, but 1 of these cases also had Grade 2 alcoholic fatty infiltration of the liver.

Of five cases dying in the malignant phase of essential hypertension, all showed lesions superficially resembling those of diabetic nephropathy. One of six cases of traumatic fat embolism showed a lesion resembling the Kimmelstiel-Wilson type, but this case also had focal necrotizing pancreatitis, which itself was probably due to the fat embolism occasioned by femoral fractures.

From the 767 autopsy cases available for study there were only 6 cases of chronic glomerulonephritis. Of these six cases, one showed renal lesions resembling those found in diabetic glomerulonephropathy.

Two of our cases of nondiabetic glomerulonephropathy merit a rather detailed de-

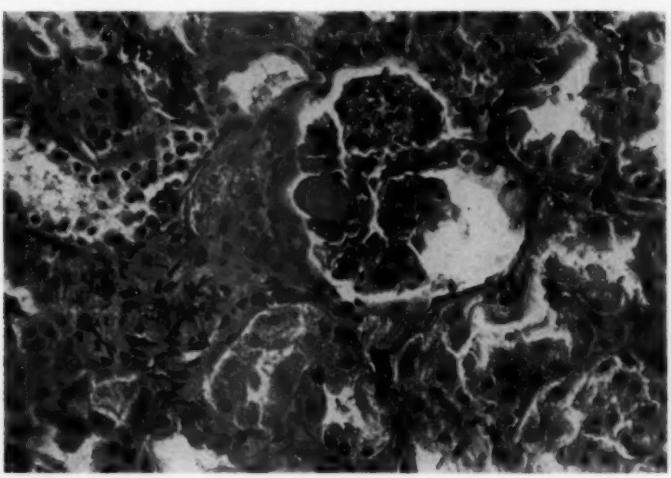

Fig. 1 (Case 3).—Exudative lesion from a case of necrotizing pancreatitis; reduced about 40% from mag. × 300.

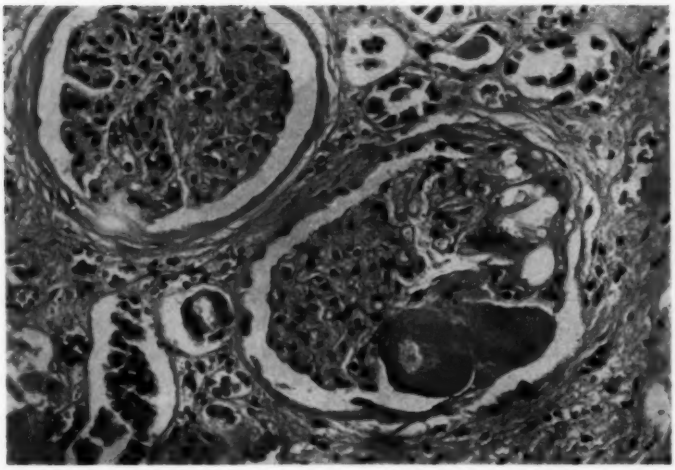

Fig. 2 (Case 1).—Glomerular lesions, capsular thickening, and tubular atrophy; reduced about 40% from mag. × 300.

scription, as the disease processes uncovered in their autopsies contribute much to the understanding of these renal lesions.

Case 1.—Acute glomerulonephropathy with pancreatitis and alcoholic fatty liver. A 38-year-old brewery owner was first hospitalized in September, 1954. He had been drinking heavily and was confused and disoriented. At that time he showed a sulfobromophthalein (Bromsulphalein) retention of 25% after half an hour; total serum protein of 7.2 gm., with 4.44 gm. albumin per 100 ml.; icterus index of 6.5 units; Kahn test negative; urine contained no sugar or ketones, with specific gravity of 1.021 and 4+ proteinuria. Blood N. P. N. was 42 mg. per 100 ml. He was treated as an alcoholic and discharged in a satisfactory condition. His second, and final, admission was in July, 1956. He had been drinking very heavily for six months prior to this, and he was extremely confused and irrational and had gross tremor of his hands. His face, neck, back, genitalia, and lower limbs were edematous. He was cyanosed and dyspneic. His abdomen was pendulous and soft but tender in all areas. Position sense was partly lost; the reflexes were absent from the extremities, and there was muscle tenderness, especially in the lower extremities. Repeated urinalyses showed a specific gravity of around 1.010, proteinuria 2+ to 3+, and no sugar or ketones. His blood showed Hb. 6.8 gm. (46%); hematocrit 22%; E. S. R. 72 mm. (Wintrobe, uncorrected); WBC 8100 per cubic millimeter. Smears showed that the red blood cells were slightly macrocytic. The serum bilirubin was 0.95 mg. per 100 ml.; total protein 5.6 gm.; albumin 3.4 gm.; globulin 2.2 gm.; γ-globulin 0.58 gm. per

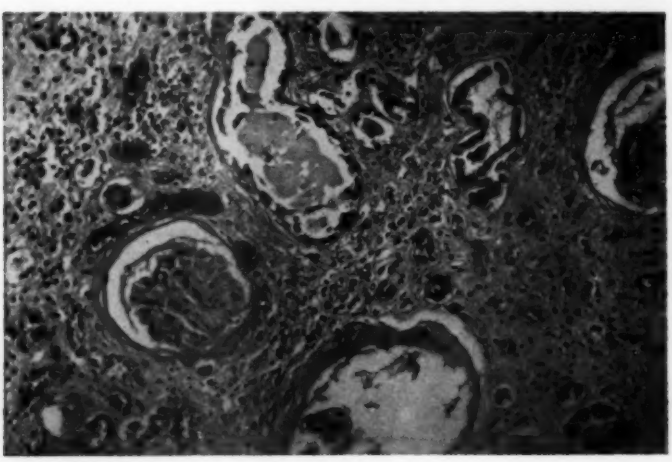

Fig. 3 (Case 1).—General view of tubular atrophy. Most of the nuclei seen represent remains of epithelial cells; reduced about 40% from mag. × 180.

100 ml.; alkaline phosphatase 26.5 King-Armstrong units; thymol turbidity 2 units; N. P. N. 86 mg. per 100 ml.; serum cholesterol 275 mg. per 100 ml.; serum Na 138.5 mEq. per liter; K 5.12 mEq.; Cl 100 mEq. per liter. The blood pressure was 130/80. The patient's condition worsened, and he died on the seventh day in the hospital. In the 12 hours prior to death his oral temperature rose to 105 F. Autopsy revealed buffalo-type obesity, flabby heart, bilateral pleural transudates (300 ml.), foci of early acute bronchopneumonia, and large fatty and cirrhotic liver. The whole pancreas was of a tan color and showed many small cystic areas filled with soft slightly granular black or brown material and was surrounded by chalky fat necrosis, which was also present in the mesentery. Both kidneys were large and pale; their capsules stripped easily, revealing pale yellowish-brown mottled surfaces. The cut surfaces showed blurring and pallor of the cortices and medullae. Tiny (barely visible) cysts were seen throughout the cut surface.

Sections confirmed the gross findings in the organs mentioned, and the kidneys (Figs. 2 and 3) demonstrated extensive exudative Kimmelstiel-Wilson type glomerulonephropathy with severe secondary tubular atrophy. Stains for amyloid were negative. Oil red O staining of frozen sections showed heavy fat embolization in lungs, brain, kidneys, and myocardium.

CASE 2.—Acute thromboembolic glomerulonephropathy. A 46-year-old beer salesman had for years consumed large quantities of beer and liquor and first sought medical attention in 1954, with weakness, nausea, and tremor of the hands. His liver was enlarged and firm at that time. He was given dietary treatment and advised to abstain from alcohol. He was again seen in June, 1956, when he complained of impotence. On the night of Dec. 26, 1956, he was found by his daughter in an epileptiform convulsion and was immediately brought to the hospital. His family said that he had been drinking "moderately" over Christmas and that he had vomited several times during the past few days. On examination his state of consciousness was greately clouded, and he grumbled of severe back and substernal pain. During the first few hours in the hospital he had three major epileptiform seizures and became delirious. Investigations revealed Hb. 16.2 gm.; WBC 13,200 per cubic millimeter, with 82% polymorphonuclear neutrophils; blood urea nitrogen 4 mg. per 100 ml.; serum calcium 9.4 mg.; blood sugar 230 mg.; urine pH 9.0, specific gravity 1.029; trace of protein; glucose 4+ and ketonuria 3+. The urine also contained small amounts of porphobilinogen, moderate amounts of uroporphyrin, and a large excess of urobilinogen. A random blood sugar value on the second day in the hospital was 145 mg. per 100 ml. Clouded consciousness merged into irritable coma, and the patient died on the third day in the hospital with high fever (104 F). Autopsy disclosed an enlarged fatty liver; diffuse hemorrhages in the intercostal muscles, diaphragm, and both psoas muscles; kidneys slightly enlarged and pale; bilateral foci of acute bronchopneumonia, and a moderate excess of cerebrospinal fluid, with corresponding generalized slight shrinkage of the brain.

Routine sections showed Grade 3 fatty infiltration of the liver with abundant fatty cysts. In the kidneys a number of glomeruli showed early thrombotic lesions (Fig. 4).

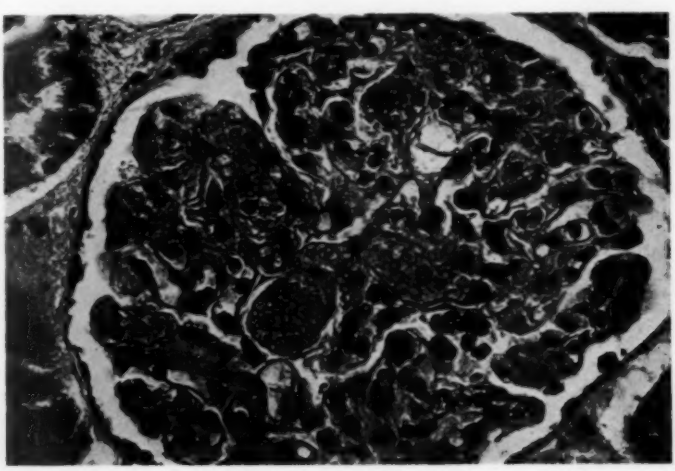

Fig. 4 (Case 2).—Early thrombosed ectasias and aneurysm; reduced about 40% from mag. × 630.

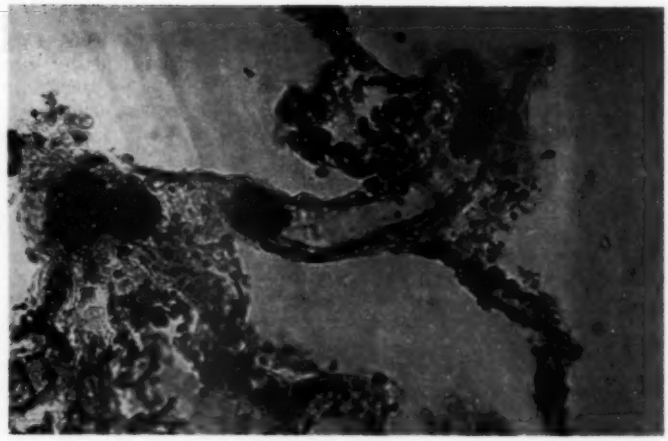

Fig. 5 (Case 2).—Lung. Fat emboli. Oil red O; reduced about 40% from mag. × 300.

Fat studies disclosed extremely heavy fat embolization in the lung, with smaller degrees of embolization throughout the brain and kidneys. In the lungs (Fig. 5) the embolization was so heavy in parts that infarction and gangrene had resulted. The combination of this embolization with liver-conditioned disorders of hemostasis was considered to be the cause of the diffuse muscle hemorrhages.

Comment

Kimmelstiel-Wilson Lesions in Necrotizing Pancreatitis and Fatty Liver.—One unifying feature in the occurrence of Kimmelstiel-Wilson-like lesions in cases of alcoholic fatty liver, cirrhosis, and necrotizing pancreatitis appears to be that of fat embolism. Hartroft has clearly described the occurrence and development of fat cysts in the livers of alcoholics and has shown beyond reasonable doubt that these cysts may rupture into bile canaliculi and/or vascular channels.[11,12] One of us has already shown that heavy fat embolization of the entire vascular system may occur in acute hemorrhagic pancreatitis, and the present investigation further substantiates this. Hartroft has further shown that when red blood cells rupture into fat cysts a peculiar mixture of fat and hemoglobin is formed, in which the break-down of the latter is impeded in

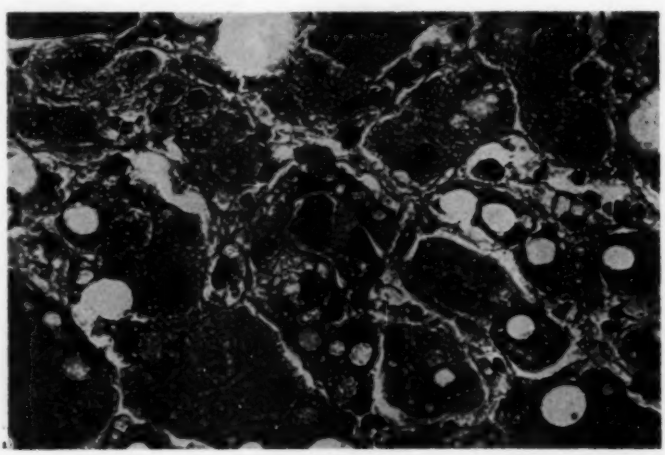

Fig. 6 (Case 2).—Hartroft lesion in liver. A globule of hemoceroid occupies most of the fat vacuole in the center of the picture; reduced about 40% from mag. × 630.

some way.[11,14] Hartroft equates this lipohemoglobin mixture to ceroid and calls it "hemoceroid."[14] Our own observations in the current work lead us to concur fully with Hartroft's deductions, and, indeed, we have come to recognize this "hemoceroid" almost regularly in alcoholic fatty and cirrhotic livers. It is seen in routine hematoxylin-and-eosin-stained sections as smooth hyaline eosinophilic globules or irregular masses within the remains of fat cysts, which to the casual or untrained observer are obscured by its presence (Fig. 6). It does not give a dissimilar appearance to Mallory bodies,[15] which are found in cells with fatty cytoplasm. Further, we have noted that such hemoceroid-filled cysts are found most frequently not where fat or fat cysts are most abundant but rather in the less fatty areas of liver and are thereby easily missed in routine sections. We assume that this observation is explained by the trauma to the relatively isolated cyst by the surrounding and more unyielding normal liver cells. The hemoceroid masses or globules seen within the remains of the fat cysts stain intensely with the benzidine and Nadi techniques, thus offering further proof of their hemoglobin content. Combined benzidine and oil red O staining give final and convincing testimony for the validity of Hartroft's observations.

Hartroft and Ridout[11] mention that they have found embolic fat in the vessels of the lungs and kidneys of a number of human cases of alcoholic fatty livers but do not give percentages. Our own findings would appear to suggest that fat embolization occurs frequently and in a high percentage of alcoholic fatty and cirrhotic liver cases and that the embolization may be very severe. It is evident from the relative numbers of emboli in lung and kidney how well the lungs function as an excretory filter for such emboli. Heavy pulmonary embolization undoubtedly accounts for the cyanosis and dyspnea so commonly seen in these cases. It is worthy of note at this juncture that both Cases 1 and 2 were in a state of delirium and irritable semicoma and that the brains of both cases showed widespread capillary fat embolization. It is not unreasonable to expect that many of the acute mental deviations of alcoholics may be explained partially on the basis of fat embolization as well as in the metabolic upsets of liver failure or other effects of alcohol. Indeed, we have instituted a clinicopathologic study to ascertain if this may not be so, and the preliminary findings are most encouraging.[16]

In acute hemorrhagic pancreatitis and in focal necrotizing pancreatitis it appears more than likely that much of the fat gaining access to the vascular channels (Fig. 7) will be admixed with both whole and lysed red blood cells and other products of tissue destruction, including some activated enzymes

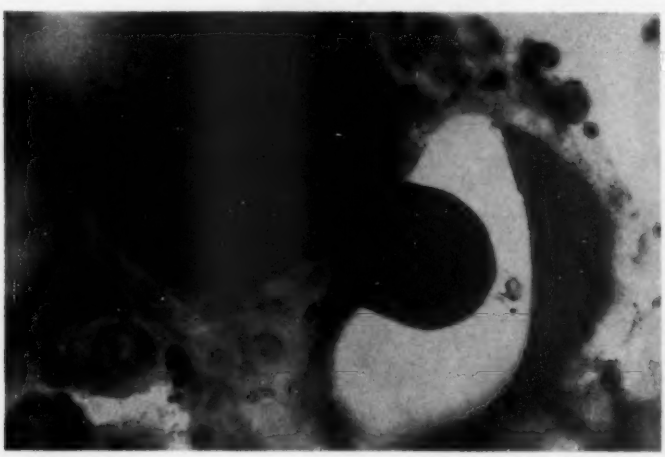

Fig. 7 (Case 4).—Fat embolization occuring in a seminecrotic area of pancreas. Sudan IV and Mayers hematoxylin; reduced about 40% from mag. × 1260.

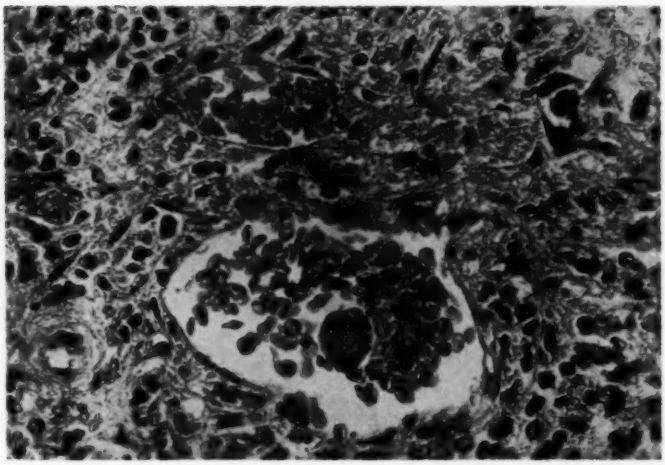

Fig. 8 (Case 5).—Hemoceroid and possibly also fatty acid emboli in blood vessels of kidney and spleen; reduced about 40% from mag. × 300.

(Figs. 8 and 9). Here it is pertinent to point out that Case 5 (which is depicted in the illustrations) was a diabetic and that we are not claiming that the majority of renal lesions of this case have any etiology other than diabetes. However, the case is included because of the great prevalance of embolic material seen in ordinary paraffin sections, because necrotizing pancreatitis (albeit microscopic) did exist, and because fat embolization was demonstrated (Figs. 8 and 9). Such emboli, should they lodge in a glomerular tuft, may be expected to produce, or to initiate the development of, occasional Kimmelstiel-Wilson-like lesions seen by us in cases of pancreatitis and of alcoholic fatty and cirrhotic livers. If such embolization is severe we may expect death or the development of severe nondiabetic Kimmelstiel-Wilson-type nephropathy as exemplified by Case 1. Our present study has revealed that lesions of the Kimmelstiel-Wilson type were more frequently and more consistently present in the kidneys of cases of necrotizing pancreatitis than in those from cases of alcoholic fatty liver disease. Also, we have noted, at least in the cases of acute hemorrhagic pancreatitis, that fat embolization is much heavier and more widespread than in cases of fatty liver; indeed, it may

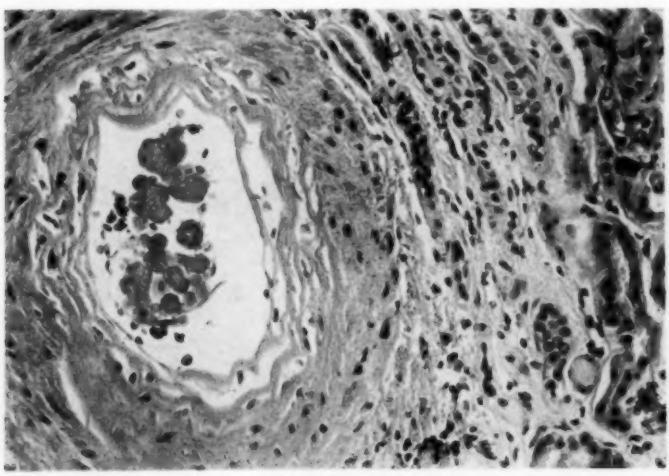

Fig. 9 (Case 5).—Hemoceroid and possibly also fatty acid emboli in blood vessels of kidney and spleen; reduced about 40% from mag. × 630.

surpass traumatic fat embolism in severity. This may account for the greater incidence of renal lesions in cases of necrotizing pancreatitis, but we feel that an additional factor here is the mixture of embolic material with activated enzymes and noxious products of tissue destruction. When these emboli lodge in a glomerular capillary they will almost certainly lead to ischemic weakening of the walls, possibly followed by localized aneurysmal dilatation. In such an environment, the embolus may then be added to by peripheral thrombotic encrustation. The relatively high lipid content of these lesions gives their characteristic glassy appearance. In this we agree with Anderson [17] when he states that the exudative lesion differs from the nodular one only in degree, and we believe that this "degree" refers to lipid content. We do not, however, wish to imply that the exudative type of lesion is always embolic. It is, we feel, often embolic in the nondiabetic cases, and while it may, in diabetes mellitus, be occasionally embolic, and then possibly on the basis of fatty hepatic cysts,[18] it is probably more often explicable on the basis of lipidic thrombi from a locally concentrated lipemic plasma [18] and other factors. Figure 4 exemplifies another possible mode of formation of some of the renal lesions. This type of early lesion appears to develop by local thrombosis in a glomerular capillary rendered ectatic probably by alcoholic toxic-anoxic effects. Homogenization of such a lesion by lysis of red blood cells, concentration of plasma lipids, and unmasking of erythrocytic lipid, we believe, leads to the development and maturation of the nodular-like lesion.

These observations are paralleled by certain findings recorded in the literature. Anderson [17] has implied that fat emboli may play a part in the genesis of some of the lesions, and Hartroft [18] and Kent [19] were of similar opinion. However, from our own limited experience we are unable to agree that fat embolization occurs commonly or heavily enough in diabetes to account for the widespread glomerulonephropathy of this disease, and we feel that diabetic nephropathy is at least partly based on erythrolipidic thrombi occurring in microaneurysmal capillaries of the glomerular tufts.[10] Relevant to our nondiabetic cases, it is noteworthy that Horn and Smetana [7] found early "intercapillary" glomerulosclerosis in a case of cirrhosis of the liver. In support of the occurrence of fat embolization in acute hemorrhagic pancreatitis, it is recorded by Simonds and Lange [20] that the most marked case of lipid deposits in the glomerular capsule and tubular epithelium seen by them was one of this disease. The work of these observers, together with that of Fuller,[21] shows that the degree of renal tubular epithelial fat deposits depends on escape of lipid through damaged glomeruli and is proportional to the degree of glomerular damage, be that induced by anoxia or by toxic substances. Our own observations from fat stains on 250 consecutive autopsies support this, and it is germane to this discussion to record our finding that the amount of fat demonstrable in the renal tubular epithelium of cases of diabetic glomerulosclerosis was not grossly excessive or remarkable and in no way comparable to the extremely high fat content of these structures in necrotizing pancreatitis. The degenerative tubular changes and hyaline thickening of the glomerular basement membranes observed by Baxter and Ashworth [22] in 25 cases of portal cirrhosis may, at least in part, be attributable to the effects of repeated minor fat embolization.

In connection with our Case 2, we wish to draw attention to the high blood sugar level found on admission to hospital and to the fact that this had fallen to a not unreasonable level two days later. This acute carbohydrate derangement we have occasionally seen in acute bouts of alcoholism and have attributed to a combination of acute depression of islet-cell function and the effects of fatty infiltration of the liver. A similar but usually severer disturbance of carbohydrate metabolism is widely recognized in acute hemorrhagic pancreatitis.

However, these are usually only temporary diabetic states and do not warrant a diagnosis of diabetes, as the sugar tolerance returns to normal after a short course of dietary treatment (in the case of acute alcoholism).

It remains to be said that in some of our cases of focal or diffuse pancreatitis we can not be certain to what extent the associated fatty livers contributed to the fat embolization. Thus, in Case 1 it is possible that emboli from the liver initiated the diffuse pancreatitis, which then, undoubtedly, played the dominant role in embolization and nephropathy development. A somewhat similar order of events may have taken place in two other cases. Likewise, in Case 9 it is felt likely that the fat embolization from fractured femora almost certainly caused a focus of necrotizing pancreatitis. Our own frozen section studies of the pancreas in such cases have shown that heavy embolization can occur from relatively small areas of necrosis. The size of the emboli, the sites of their arrest in the pulmonary and other circulations, and, presumably therefore, their demonstrability in organs such as the kidney appear to depend largely on the extent of tissue damage and the presence or absence of shock.[23] Again, failure to demonstrate emboli in cases of fatty liver or in cases of pancreatitis having its onset weeks or months before death occurs does not mean that fat embolization did not take place in such cases, as the emboli may have been eliminated by the time a search is made for them.[19]

Kimmelstiel-Wilson Lesions in Chronic Glomerulonephritis.—Allen [3] found in 5 of 34 cases of chronic glomerulonephritis "lesions that might be regarded as suggestive of the diabetic change." However, he stressed that large lesions were never found in this disease; rather, they were "uniformly small and arranged with a characteristic lobularity." Allen's observations fitted our case well (Fig. 10).

Kimmelstiel-Wilson-like Lesions in Essential Hypertension.—At the outset we wish to state that in no case of hypertension did we see a lesion unequivocally resembling the Kimmelstiel-Wilson-type, save in one (Case 10), where there was a Grade 2 fatty infiltration of the liver. In that case we saw one "blob" lesion, but this may have had its origin in an embolus from the fatty liver. The hilar lesions seen in hypertension were almost all connected with the hyaline thickening of the afferent arteriole. Generally speaking, the pseudo-Kimmelstiel-Wilson lesions of hypertension are of irregular outline, more often hilar or juxtahilar in position, granular rather than hyaline, and usually are obviously due to the rhexis of an

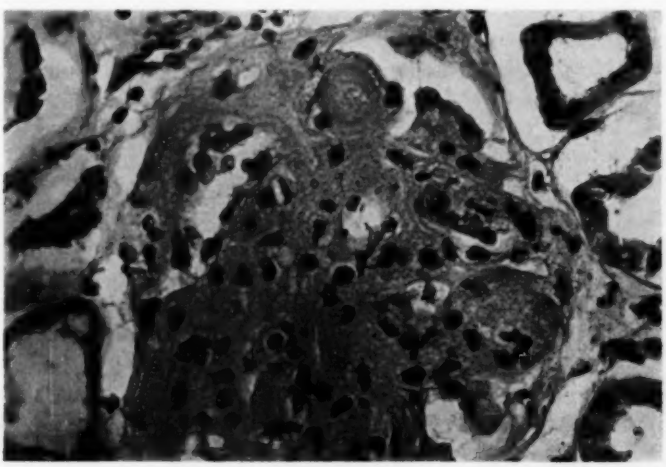

Fig. 10 (Case 6).—Chronic glomerulonephritis; the small nature of the lesions is evident; reduced about 40% from mag. × 630.

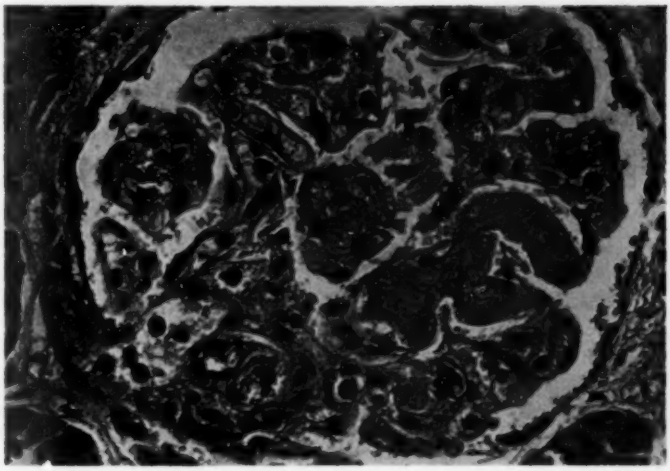

Fig. 11 (Case 7).—Granular capillary lesion. Benign hypertension; reduced about 40% from mag. × 630.

acute destructive physical process. Benzidine and Nadi staining show that microaneurysms are rare in hypertensive arteriolonephrosclerosis but that irregular vascular disruptive processes are common.[10] We feel that such lesions can easily be distinguished from true Kimmelstiel-Wilson lesions even in routine hematoxylin-and-eosin-stained sections (Figs. 11 and 12).

Summary

Evidence has been adduced to show that the Kimmelstiel-Wilson lesion is not absolutely specific for diabetes mellitus.

Occasional lesions of this type were found in the kidneys of 12 of 20 cases of necrotizing pancreatitis (diffuse or focal). In nine of these the lesions were abundant enough to indicate a causal relationship, while in one case they were as extensive and abundant as in the severer cases of diabetic glomerulosclerosis.

Of 33 cases of alcoholic fatty infiltration of the liver, 6 showed occasional Kimmelstiel-Wilson lesions.

In 23 instances of alcoholic portal cirrhosis, 4 showed early or slight renal lesions.

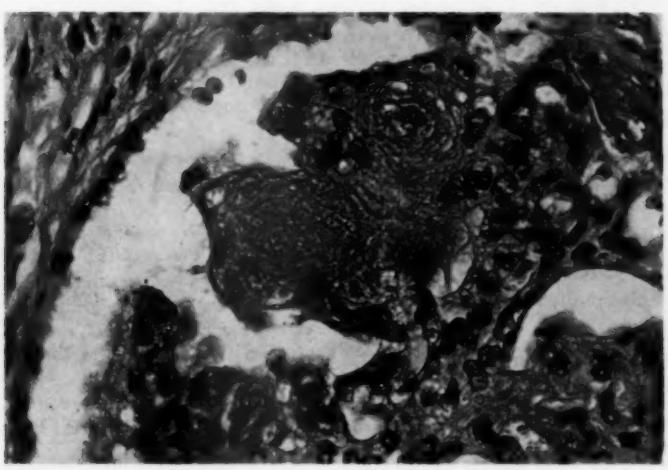

Fig. 12 (Case 8).—Malignant hypertension. The destructive granular nature of the lesion is evident.

The etiology of the lesions in such cases is felt to be on the basis of embolization by mixtures of fat and lysed red blood cells (hemoceroid) derived from Hartroft fat cysts of the liver and from necrotic areas of pancreas.

Generally, the renal lesions are more numerous and more marked in pancreatitis, and this is believed to be due to heavier embolization from this source, together with the enhancing effect of active enzymes and necrotic tissue products. However, it should be emphasized that in the cases of pancreatitis the lesions were always relatively scanty, except in Case 1, in which it is believed there was aggravation of heavy fat embolism from the liver by recurrent attacks of pancreatitis.

Evidence of generalized embolization was further culled from the lungs of 11 of 20 cases of focal or diffuse pancreatitis: in 13 of 24 cases of fatty liver, and in 6 of 11 cases of alcoholic cirrhosis investigated by oil red O staining. In one case of alcoholic fatty liver, fat embolization was as heavy as that seen in traumatic cases.

In 46 cases of benign hypertension, 4 cases showed lesions resembling the Kimmelstiel-Wilson type, while all of 5 cases of malignant hypertension had such glomerular changes. However, it is felt that the lesions seen in hypertensive kidneys can easily be distinguished from Kimmelstiel-Wilson lesions.

One of six cases of chronic glomerulonephritis showed small, but otherwise fairly typical, lesions, and benzidine and Nadi studies showed that most of these were based on small microaneurysms in the capillaries of the glomerular tufts.

All the cases of nondiabetic Kimmelstiel-Wilson type glomerulonephropathy found were of the exudative type, which may indicate the immaturity and juxta-agonal occurrence of such lesions. The study in effect emphasizes the specificity of the nodular lesion in diabetes mellitus.

Sudbury General Hospital of the Immaculate Heart of Mary, Paris St. (Dr. Lynch).

Mrs. Irena B. Eley, S.T., R.T., prepared the sections, and Mr. L. D. Mellor, F.I.M.L.T., S.T., R.T., did the biochemical investigation of the cases reported here.

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Carcinoma of the Anorectal Junction with "Extramammary Paget's Disease"

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We have recently had an opportunity to study a patient with an unusual type of carcinoma of the anorectal junction which presented features of "extramammary Paget's disease." Consideration of this case in relation to the recent detailed histological studies of the anorectal junction by Grinvalsky and Helwig [1] is of interest in regard to the problem of the histogenesis of those lesions of the perianal area that resemble Paget's disease of the breast.

Report of Case

The patient, a 67-year-old white man, was admitted to the Clinical Center of the National Institutes of Health on Jan. 16, 1956, for evaluation of "perianal precancerous changes." In 1943, he had received multiple injection treatments for hemorrhoids and during the subsequent six years had no rectal difficulties. In 1949, he complained of perianal itching, and anal tags were noted. A hemorrhoidectomy was performed, and no evidence of tumor was found in the excised tissues.

After the operation, he had rectal pain and perianal bleeding. In 1950, he was found to have thickened excoriated perianal skin. Sigmoidoscopy revealed no significant abnormalities other than excessive colonic mucus. No lesions were found radiologically after a barium enema.

He continued to have perianal itching, and in 1952 a diagnosis from a biopsy specimen of the perianal region was reported as "epithelial hyperplasia with dyskeratosis and parakeratosis."

The patient was seen by a dermatologist, who felt that the clinical features suggested a diagnosis of extramammary Paget's disease. On review of the histological sections, large atypical cells resembling Paget's cells were noted. The entire area of involved skin and mucosa was excised in October, 1952, and diagnosed histologically as "precancerous changes in perirectal tissue." Large atypical vacuolated cells were again seen in the surface epithelium and were thought to resemble Paget's cells, although no involvement of underlying glands could be found. In November, 1953, this patient was the subject of a case report titled "Precancerous Perianal Lesion." [2]

After the excision of the perianal tissues in 1952, rectal pain and itching continued and increased in severity; perianal lichenification, erythema, and maceration were noted. In December, 1955, the patient was referred to the Clinical Center for further clinical investigation and therapy.

Physical examination at the time of admission revealed considerable maceration, redness, fissuring, and thickening of the perianal skin, as illustrated in Figure 1. Other findings, apparently unrelated to the anal lesion, were pulmonary emphysema and inactive pulmonary tuberculosis, pulsion divertic-

Fig. 1.—Photograph of patient's anal region. Thickening and fissuring of perianal skin can be seen.

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Laboratory of Pathology (Dr. Rabson), General Medicine Branch (Dr. Van Scott), and Surgery Branch (Dr. Smith), National Cancer Institute, National Institutes of Health, Public Health Service, U. S. Department of Health, Education and Welfare.

ulum of the lower esophagus, a small esophageal hiatus hernia, chronic maxillary and ethmoid sinusitis, multiple nodules of the thyroid gland, and osteoarthritis of the cervical spine. The serological test for syphilis was weakly reactive; there was a history of treatment for syphilis 45 years previously.

Sigmoidoscopy shortly after admission showed thickened gray-white anal mucosa, with areas of excoriation and fissure formation extending upward to the anorectal junction. There was no evidence of infiltrative tumor. The rectal mucosa above the hemorrhoidal line appeared normal.

Three surgical procedures were performed during his hospitalization. These included a punch biopsy of perianal skin on Jan. 23, local excision of anal skin and mucosa on Feb. 16, and an abdominal-perineal resection of the rectosigmoid and rectum on March 9.

After the last operation the patient was discharged and has been followed in the clinic. He has had no perineal symptoms, and there was no evidence of recurrent tumor 15 months later.

Pathological Findings

The first specimen was a 6 mm. punch biopsy specimen of perianal skin. Microscopically, there was a surface layer of stratified squamous epithelium with moderate acanthosis. Scattered in the epithelial layer were many atypical cells with hyperchromatic pleomorphic nuclei and eosinophilic cytoplasm. A number of these atypical cells appeared to be situated within clear spaces, and in some cases the hyperchromatic nuclei were located at the margin of a clear space containing a small amount of fibrillar eosinophilic material (Fig. 2). The lesion was thought to represent an intraepithelial carcinoma, and a diagnosis of Bowen's disease was made. Subsequently, sections stained with mucicarmine and those prepared with the periodic acid-Schiff reaction after diastase digestion demonstrated considerable amounts of mucin-like material in the cyto-

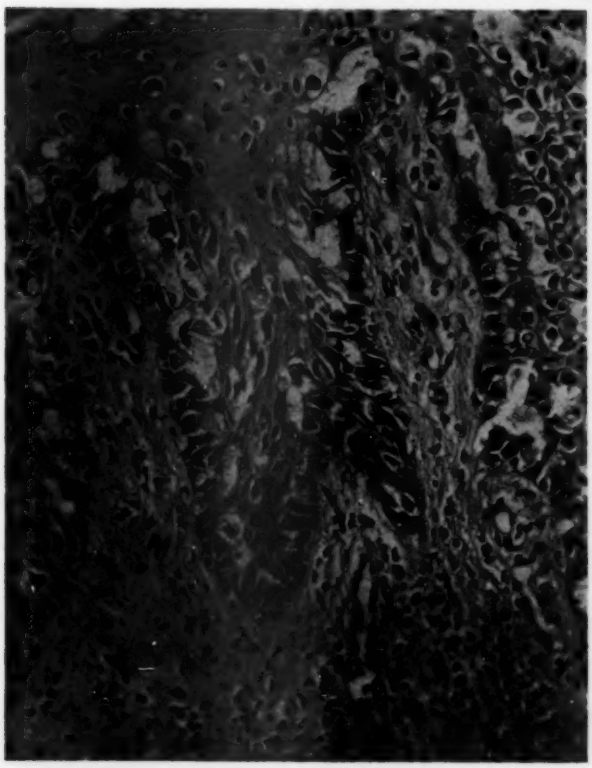

Fig. 2.—Biopsy specimen of perianal skin. Cells with hyperchromatic pleomorphic nuclei are present in the surface epithelium, and many of these seem to be within clear spaces. Mucin was demonstrated in these spaces with the mucicarmine stain and the periodic acid-Schiff reaction after diastase digestion. Hematoxylin and eosin; × 205.

Fig. 3.—Section of perianal skin, with apocrine ducts and glands involved by tumor. The ducts at the extreme right are lined by uniform columnar cells showing "decapitation secretion" (illustrated at higher magnification in Figure 6). The ducts at the left and in the center are lined by tumor cells. Extending downward from the rete pegs are gland-like structures lined by tumor cells. Hematoxylin and eosin; reduced 15% from mag. × 63.

plasm of the atypical cells and in the clear spaces described above. In view of these findings, the lesion was thought to be an example of extramammary Paget's disease, although no underlying glandular involvement could be found in the biopsy specimen.

The second surgical specimen was from the extensive resection of perianal skin and mucosa. The gross limits of the lesion were defined with difficulty. In the sections examined microscopically, atypical vacuolated pleomorphic cells similar to those described in the punch biopsy specimen were again seen in the surface stratified squamous epithelium. In addition, as shown in Figures 3 and 4, the basal layer of the rete pegs contained groups of mucus-secreting columnar cells with nuclear pleomorphism and hyperchromatism. In some areas, these columnar cells were arranged in crypts and in gland-like structures which could be seen to open into tracts in the squamous epithelium (Fig. 4).

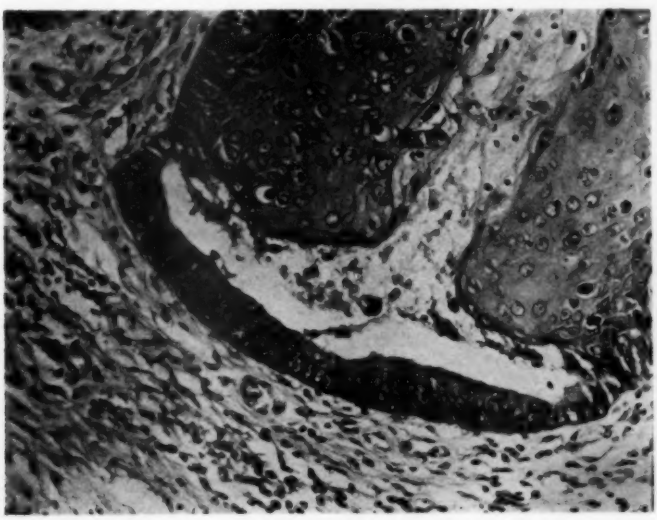

Fig. 4.—Base of rete peg of perianal skin, with downward projection of gland-like structures lined by tumor cells. There is a tract through the squamous epithelium connecting the gland to the surface of the skin. Hematoxylin and eosin; reduced 15% from mag. × 205.

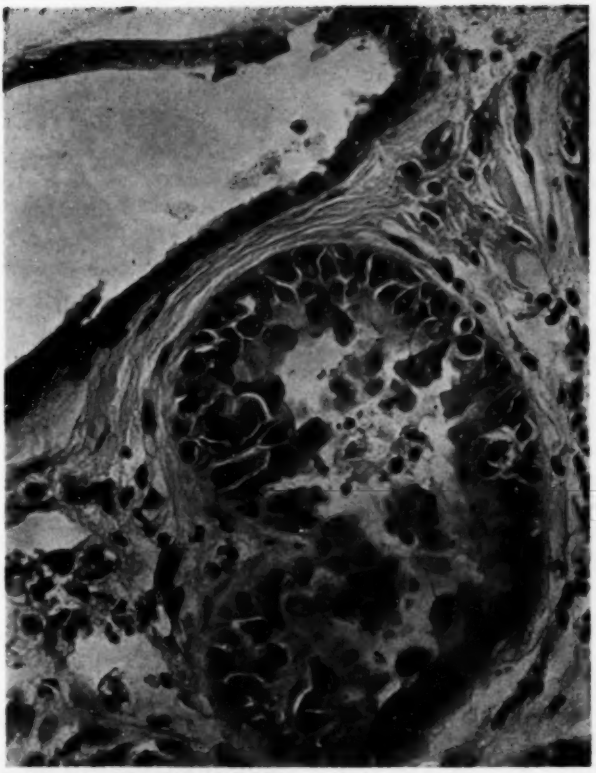

Fig. 5.—Higher magnification of apocrine ducts lined by tumor cells seen in Figure 3. The cells lining the inferior of the two ducts have pleomorphic hyperchromatic nuclei and resemble the tumor cells in the surface epithelium, as shown in Figure 2. Hematoxylin and eosin; × 525.

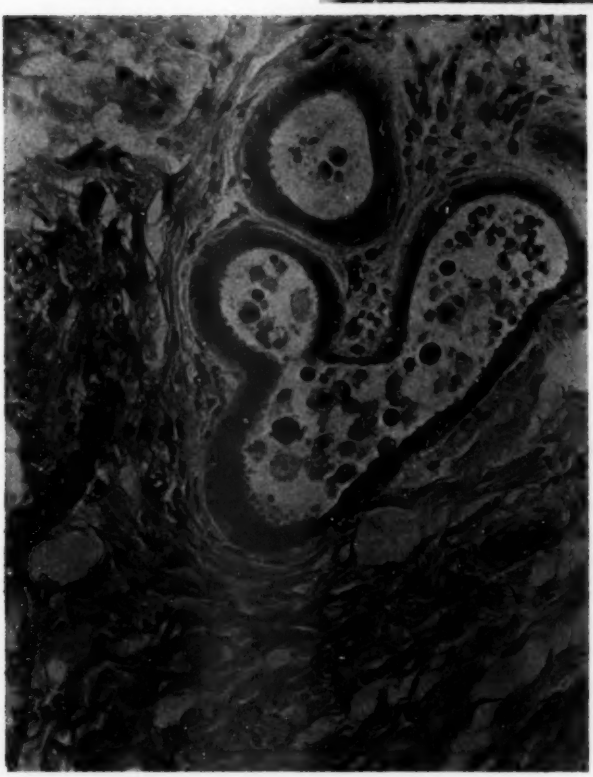

Fig. 6.—Higher magnification of apocrine gland structures present at extreme right in Figure 3. "Decapitation secretion" is evident. Leukocytes and degenerated tumor cells are present in the lumens. Hematoxylin and eosin; × 205.

In one section, apocrine glands in the deep subepithelial connective tissue had areas involved by tumor (Figs. 3 and 5). The apocrine nature of these glands was established by histological evidence of "decapitation secretion" (Fig. 6). Serial sections of this block and of several other tissue blocks from adjacent regions failed to demonstrate any additional involvement of apocrine sweat glands.

Examination of the surgical margins of the specimen showed intraepithelial tumor cells in the mucosa within 1 mm. of the proximal surgical margin. No residual tumor could be found, however, in the specimen of sigmoid colon and rectum removed 21 days later.

Comment

Extramammary Paget's disease of the perianal area has been frequently reported and discussed.[2-7,9] The histogenesis of these lesions and their relation to apocrine sweat glands, however, seem to be unsettled. Allen, in his recent monograph on dermatopathology, has stated that "the evidence that the entity 'extramammary Paget's disease' of the skin derived from sweat glands exists must be seriously doubted." [8]

In our own case, involvement of apocrine type sweat glands by adenocarcinoma in the presence of intraepidermal tumor cells resembling Paget's cells has been demonstrated. Nevertheless, it is difficult to attribute the extensive distribution of tumor cells in the surface epithelium to extension or metastasis from a primary tumor in apocrine sweat glands.

The recent detailed histological studies of the anorectal junction by Grinvalsky and Helwig demonstrated mucus-containing columnar and goblet cells in the transitional-cell epithelium of the normal anorectal junction. They also found numerous mucus-containing cells lining anal ducts and glands.

It is our opinion, therefore, that the present case is an adenocarcinoma of the anorectal junction with origin in multiple sites. These include mucus-secreting cells of the surface epithelium and anal ducts and glands and an apocrine sweat gland. This is in accord with the concept that mammary Paget's disease represents origin of tumor both in mammary ducts and in cells of the epidermis rather than that Paget's cells are the result of upward migration of the cells of a primary duct carcinoma of the breast.

It is of interest that the tumor cells, the normal mucus-secreting cells of the anorectal junction, and the cells of apocrine glands all have intracytoplasmic material which stains with mucicarmine and which is periodic acid-Schiff-reactive after diastase digestion. The diagnostic importance of these findings has been emphasized by Dockerty and Pratt [3] in their report on extramammary Paget's disease, and in the present case, the nature of the lesion and its differentiation from Bowen's disease were more clearly established by the use of such stains.

Summary

A case of adenocarcinoma of the anorectal junction with features of extramammary Paget's disease is reported, with apparent origin of the tumor from mucus-secreting cells of the surface mucosa, anal ducts and glands, and a perianal apocrine sweat gland. Histogenetic considerations of this lesion are discussed, and the importance of stains for mucin in establishing the diagnosis is emphasized.

Clinical Center, National Institutes of Health.

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Degenerative Joint Disease in Castrate Mice

I. Effects of Ovariectomy at Various Ages

RUTH SILBERBERG, M.D.; GEORGE GOTO, M.D., and MARTIN SILBERBERG, M.D., St. Louis

The joints of old mice undergo age changes or develop osteoarthritis. Such lesions have been repeatedly described and illustrated [1,2]; they constitute an analogue of the human disease and are strictly noninflammatory in nature, as the alternate term "degenerative joint disease" implies.[3] Articular lesions found in rodents under various experimental conditions and characterized by acute inflammatory reaction [4] or pannus formation [5] cannot be classified as osteoarthritis.

In male mice of various strains degenerative joint disease is commoner and severer than in females.[1,2] This sex difference may be due to differences in the genetic make-up of the male and female skeletons, respectively, or it may be related to the action of sex hormones; male sex hormone might promote or female sex hormones might inhibit the evolution of osteoarthritis. As to the skeletal effects of female sex hormones, those exerted by progesterone are minor as compared to those exerted by estrogens.[6] The skeletal effects of ovariectomy would thus be essentially those of estrogen deficiency. Ovariectomy performed in weanling mice attenuated the course of osteoarthritis.[6] This effect was thought to be related to the retardation of skeletal development and aging subsequent to the removal of the ovaries. Since the response of cartilage to a variety of stimuli changes with age,[6] the reaction of adult and old cartilage to ovariectomy might also differ from that of young cartilage. The present paper deals with the results of experiments carried out in order to investigate this possibility.

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From the Snodgras Laboratory, City Hospital Division, and the Department of Pathology, Washington University School of Medicine.

The investigation was supported by Research Grant A-22(C7) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.

Material and Methods

One hundred seventeen female mice of the closely inbred Strain C57BL raised in our laboratory and kept on a stock diet of Purina Laboratory Chow and water ad libitum were spayed—64 at 6 months and 53 at 12 months of age; 45 untreated females served as controls. The animals were killed when they were 18 or 24 months old unless they died prematurely or had to be killed because of sickness. At necropsy the knee joints were removed and prepared for microscopic studies as usual.[3] The articular findings were compared with those obtained previously in 45 females of Strain C57BL spayed at one month of age.[6] The statistical significance of the results was determined with the χ^2-method.

Observations

Articular age changes consist of proliferation, hypertrophy, and degeneration of cartilage or ligaments, while the surfaces remain intact. Osteoarthritic lesions may vary from slight to advanced. Changes were classified as slight if there was a combination of growth and degeneration of cartilage associated with minor roughening and fraying of the joint surface and but slight involvement of ligaments and synovialis. Changes were classified as advanced if there was marked hyperplasia, hypertrophy, or ulceration of the cartilage or a combination of both, with involvement of bone marrow, ligaments, synovialis, and capsule, causing deformities of the joint. Representative gross and microscopic findings have been repeatedly illustrated.[1,2]

Effects of Ovariectomy Performed at Various Ages

Subject	Mouse No.	Mean Age, Mo.	Intact Joints, % (No.)	Age Changes, % (No.)	Osteoarthritis		
					Early, % (No.)	Advanced, % (No.)	Total, % (No.)
Intact females	42	18.5	26 (11)	57 (24)	2 (1)	14 (6)	17 (7)
Spayed at 1 mo.	45	19.9	67 * (30)	22 (10)	9 (4)	2 (1)	11 (5)
Spayed at 6 mo.	64	18.3	77 † (49)	20 (13)	0 (0)	2 (2)	2 * (2)
Spayed at 12 mo.	53	19.7	51 (27)	38 (20)	9 (5)	2 (1)	11 (6)

* $P<0.05$.
† $P<0.02$.

The results of the microscopic examination of the knee joints are summarized in the Table. Findings in mice spayed at the age of one month [6] have been included in order to facilitate a comparison.

Intact Joints.—Twenty-six per cent of untreated mice with a mean age of 18.5 months at death had unaltered joints. In mice spayed at the age of 6 months and dead at a mean age of 18.3 months the incidence of intact joints was 77%. This almost threefold increase is statistically significant at the 2% level. It indicates that age changes as well as arthritic lesions were retarded subsequent to ovariectomy at this age. In mice spayed at the age of 12 months with a mean age of 19.7 months at the time of death 51% of the knee joints were unchanged. The effect of castration performed at 12 months of age was thus less marked than it was in mice spayed at the age of 6 months. It is unlikely that the slightly higher mean age at death accounts for this difference.

Age Changes.—The increased number of intact joints subsequent to ovariectomy was associated with a decrease in the incidence of age changes. This decrease was likewise more conspicuous in mice castrated at 6 months of age than in those spayed at the age of 12 months.

Osteoarthritis.—In untreated females of Strain C57BL the incidence of osteoarthritis was 17%. Ovariectomy was most effective in attenuating the course of osteoarthritis if it was performed at 6 months of age.

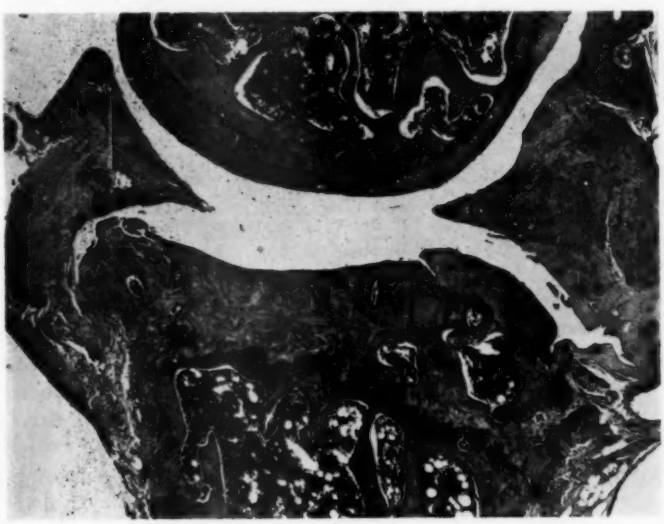

Fig. 1.—Spontaneous osteoarthritic ulcers in tibia and femur. Femur in upper and tibia in lower aspect of the photomicrograph; reduced 10% from mag. × 45.

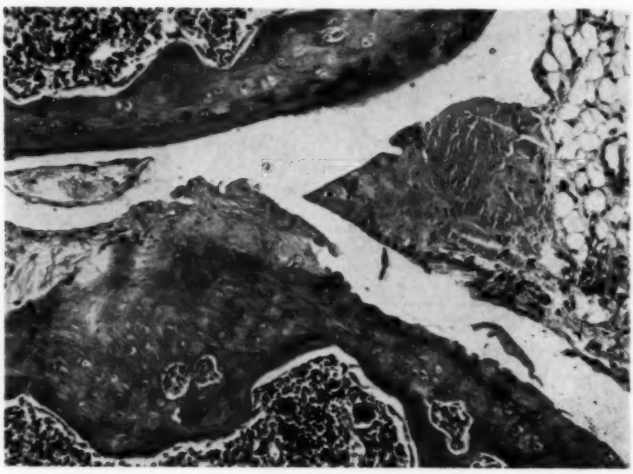

Fig. 2.—Shallow erosion in tibia seen after ovariectomy. Orientation as in Figure 1; × 125.

Under these conditions the incidence of the disease went down to 3%. This drop is statistically significant at the 5% level. Of mice spayed at either 1 or 12 months of age about 11% had osteoarthritis, a statistically not significant decrease from the incidence seen in untreated controls.

The attenuating effect of ovariectomy on the course of degenerative joint disease appears more impressive than the statistical data indicate if the severity of the lesions is taken into account. Six of seven untreated arthritic mice showed advanced joint lesions. By contrast only one of six arthritic mice castrated at the age of 1 month and one of six arthritic mice castrated at the age of 6 months had severe joint disease. The photomicrographs show typical osteoarthritic lesions as found in untreated and in castrated mice, respectively. The difference in severity is obvious (Figs. 1 and 2).

Comment

In female mice of Strain C57BL, ovariectomy performed at 1, 6, or 12 months retarded articular aging; extirpation of the ovaries also decreased the incidence and severity of osteoarthritis as compared with conditions in untreated controls. These findings do not corroborate the view that ovariectomy causes premature aging[7]; they are rather in line with observations made in tissues other than the skeleton and indicating that lack of sex hormones retards processes of aging.[8]

Since ovariectomy, irrespective of the age at which it was carried out, did not enhance the susceptibility to osteoarthritis, it is unlikely that in the untreated female mouse endogenous estrogens counteract the development of the joint disease. It is, therefore, also improbable that the comparatively low susceptibility of the female to osteoarthritis is due to a protective influence exerted by estrogen. The present observations seem to speak against the existence of true menopausal arthritis. However, a final answer to this question will depend upon the outcome of studies in other strains and species.

Interpretations as to the mechanisms underlying the articular effects of ovariectomy have of necessity to be speculative as long as the basic action of ovarian hormones on the skeleton is not adequately understood.[6] Articular age changes are by many investigators[3] considered to be precursors of senescent osteoarthropathy. In conformity with this view and with the findings presented, the arthritis-inhibiting action of ovariectomy may then be wholly or partly due to a delay of age changes, whatever their basic nature may be. While ovariectomy performed before sexual maturity attenuated the develop-

ment of osteoarthritis to some extent, the effect of ovariectomy was most significant in animals spayed at the age of 6 months. This fact may be due to a change in the reactivity of the cartilage with age, or after castration early in life a compensatory hormonal mechanism may develop which might restore a more physiological rate of articular aging. In a number of strains of mice the adrenal cortices take over the production of estrogen after castration.[9] In some of the mice spayed before sexual maturity, but not in the other series, hyperplastic cortical nodules were observed; no direct evidence was obtained that these adrenal nodules were functioning. However, such nodules do not always produce hormones, nor does the absence of nodules indicate absence of hyperfunction.[9] That only minute amounts of hormones could have been involved is indicated by the fact that the effect of ovariectomy was not completely suppressed.

The comparatively slight effect of ovariectomy performed at 12 months of age was according to expectation. At the time of operation, articular aging must be assumed to have already made considerable progress; ovariectomy under these circumstances could then at best slow down further deterioration of existing lesions but would not restore normal conditions in joints which had already undergone pathological changes.

Summary

In female mice of Strain C57BL, castration performed at 1, 6, or 12 months of age retarded articular aging and delayed the onset, decreased the incidence, and attenuated the course of osteoarthritis as compared to conditions seen in untreated controls. These effects were most marked in animals spayed during early adulthood. Endogenous ovarian hormones thus do not protect the joints of the female mouse against the development of degenerative joint disease. The lower susceptibility of the female to osteoarthritis as compared to that of the male can therefore not be accounted for by ovarian activity.

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Degenerative Joint Disease in Castrate Mice

II. Effects of Orchiectomy at Various Ages

RUTH SILBERBERG, M.D.; ROBERT THOMASSON, M.D., and MARTIN SILBERBERG, M.D., St. Louis

In continuation of studies of the articular effects of castration in female mice reported in the preceding paper, the response of male mice to orchiectomy was investigated.

Material and Methods

SERIES 1.—Fifty-three male mice of the closely inbred Strain C57BL were castrated at the age of one month; seventy-seven males of the same strain served as controls.

SERIES 2.—Thirty-two male mice of Strain DBA were castrated at the age of 1 month; 43, at the age of 6, and 46, at the age of 12 months; 50 males of the same strain were used as controls.

The animals were kept on a stock diet of Purina Laboratory Chow and water ad libitum. A number of mice were killed at 18 or 24 months of age; others died or had to be killed because of sickness before they had reached these ages. The mean ages at death, which varied from 17.2 to 19.6 months in Strain C57BL and from 18 to 20.5 months in Strain DBA, are given in the Table.

The knee joints were prepared for microscopic study as usual.[1,2]

Submitted for publication Oct. 28, 1957.

From the Snodgras Laboratory, City Hospital Division, and the Department of Pathology, Washington University School of Medicine.

The investigation was supported by Research Grant A-22(C7) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, Public Health Service.

Observations

Histologic criteria for age changes and osteoarthritis have been given in the preceding paper. The findings are summarized in the Table.

Strain C57BL.—Intact joints were found in 9% of the untreated controls which at the time of death had a mean age of 19.3 months. By contrast, unaltered joints were found in 34% of the animals orchiectomized before sexual maturity and 19.6 months old at the time of death. This increase is highly significant ($P<0.001$). It indicates a delay in the onset of the articular lesions. Correspondingly, the incidence of osteoarthritis in the castrates dropped from 43% in the controls to 6% in the castrates. This decrease is likewise highly significant ($P<0.001$). There was at the same time

Effects of Orchiectomy Performed at Various Ages

Subject	Mouse No.	Mean Age, Mo.	Intact Joints, % (No.)	Age Changes, % (No.)	Osteoarthritis, % (No.)
Strain C57BL					
Intact males	77	19.2	9 (7)	48 (37)	43 (33)
Castrated at 1 mo.	53	19.6	34 * (18)	60 (32)	6 * (3)
Strain DBA					
Intact males	50	18.0	6 (3)	64 (33)	30 (15)
Castrated at 1 mo.	32	19.4	19 (6)	75 (24)	6 † (2)
Castrated at 6 mo.	43	20.5	5 (2)	65 (28)	30 (13)
Castrated at 12 mo.	46	20.0	15 (7)	59 (27)	26 (12)

* $P<0.001$.
† $P<0.03$.

a striking attenuation of the lesions. Large ulcers with eburnated floors, which are not infrequent in untreated animals, were not seen at all in the castrates; instead there were only shallow erosions of the articular surface. There was an apparent slight increase in the number of animals showing age changes. However, this finding has to be evaluated in relation to the decrease in the incidence of osteoarthritis. Since osteoarthritis is preceded by age changes,[3] a number of lesions that would in noncastrate animals progress to the arthritic stage fail to do so in the castrate, and thus a numerical increase in age changes results. This finding must therefore not be construed as an age-accelerating effect of castration.

Strain DBA.—Castration performed at the age of one month increased the incidence of intact joints from 6% to 19%, an effect which was less conspicuous than that in Strain C57BL and statistically not significant. The incidence of osteoarthritis, on the other hand, decreased from the 30% seen in the untreated controls to a low of 6% in the castrates. This difference is statistically significant at the 3% level. As far as age changes are concerned the same considerations apply as for mice of Strain C57BL. The slight numerical increase which was noted in the castrate should be correlated to the simultaneous decrease of arthritic lesions.

Castration carried out at 6 or 12 months of age did not affect the incidence of osteoarthritis, which remained about the same as in the untreated animals. The joint lesions were less severe than in noncastrates. However even in untreated mice of Strain DBA osteoarthritis takes a milder course than in mice of Strain C57BL, A, or YBR/Wi.

Comment

In male mice of Strain C57BL and DBA, orchiectomy performed before sexual maturity retarded the onset and progress of articular age changes. In mice of both strains the incidence—and in Strain C57BL also the severity—of osteoarthritis was markedly decreased as compared to findings in untreated controls. Orchiectomy carried out in early or late adulthood had only limited or no effect on the joints.

Male sex hormone must therefore be assumed to act on the articular cartilage at an early age, that is, the phase of active skeletal growth. Lack of testicular hormone during this period has been shown to result in retardation of growth at the epiphyseal plates and delay of skeletal development and aging.[2,4,5] Administration of male sex hormone to hypogenital[2,5-7] or castrated individuals,[2,5,7,8] on the other hand, promotes growth and aging of the epiphyseal cartilage. Obviously, the growth-stimulating effect of male hormone is related to its protein-anabolic action[2,5,9] and the growth-inhibiting effect of orchiectomy, to lack of this action. If conditions in the articular cartilage simulate those in the growth zones—which is likely in view of the histogenetic identity of the two tissues—the sequelae to early castration would be inhibition of growth and development and delay of aging of cartilage followed by retarded evolution of osteoarthritis. Ultimately the attenuation of degenerative joint disease in the male castrate may thus result from a deficiency of growth caused by lack of testicular hormone.

While orchiectomy had its most striking effect if performed before sexual maturity, ovariectomy as reported in the preceding paper delayed articular aging and the development of osteoarthritis most conspicuously if carried out in early adulthood. This correlates well with differences in the effects of male and female sex hormone on growing epiphyseal cartilage. Unlike androgens, estrogens do not promote growth but enhance processes of aging.[2,5,10] This explains why in the joints also lack of female sex hormone manifests itself not so much during the period of growth as after cessation of growth.

A comparison of the articular effects of orchiectomy and ovariectomy, respectively, discloses that the sex differences in osteoarthritis present in noncastrate mice are to

a large extent eliminated in the castrates. These observations point to an important role played by hormones in the different susceptibility to the disease of male and female mice, respectively.

Summary

Orchiectomy performed in sexually immature male mice of Strains C57BL and DBA significantly delayed articular aging and attenuated the evolution of osteoarthritis as compared to the course of events in noncastrates. Orchiectomy carried out in adult male mice was less effective than castration performed early in life. The articular effects of orchiectomy are thought to be related to lack of growth stimulation by male sex hormone essential for the development of cartilage in the growing animal.

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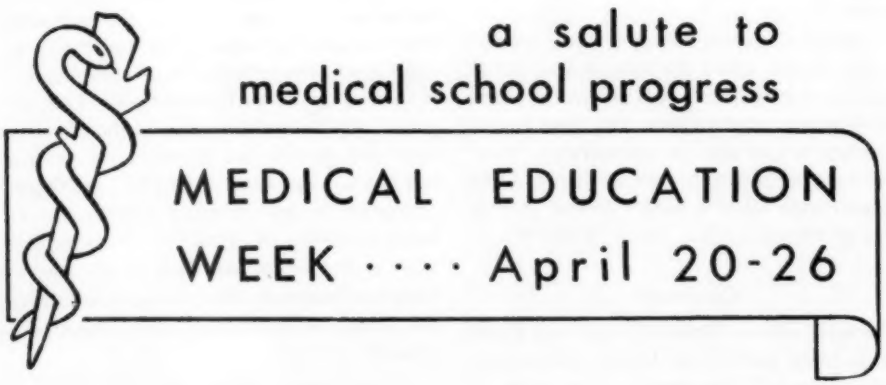

Terminal Pancreatitis

ARTHUR A. STEIN, M.D., and SAMUEL R. POWERS Jr., M.D., Albany, N. Y.

Sachar and Probstein [1] have recently called attention to the occurrence of localized acute pancreatitis as an incidental finding at necropsy. Their 10 cases were sharply differentiated from those of clinically recognizable acute pancreatitis by the absence of related signs or symptoms and the absence of any anatomical or clinical evidence of associated biliary disease. At necropsy the primary anatomical causes of death in these patients included such apparently unrelated entities as acute hepatitis and other infectious processes, cancer, and vascular accidents. These investigators were unable to identify any common denominator in these cases of acute pancreatitis but suggested vascular complications and increased intraductal pressure in the pancreas as potential contributing factors.

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In a study of the pathogenesis of acute hemorrhagic pancreatitis, the pancreases of 432 consecutive autopsies performed at the Albany Hospital during 1953 were reviewed. After exclusion of infants and those adults in which the pancreas showed marked autolysis, 358 cases remained for study. The clinical charts, the autopsy protocols, and the histologic sections from the pancreas were carefully reviewed in order to determine the incidence of pancreatitis and to search for factors and mechanisms which might be involved in the production of this condition.

On histologic review, 66% of the pancreases studied in 358 necropsies showed foci of chronic interstitial pancreatitis. This entity was manifested by focal edema and infiltrations of chronic inflammatory cells in the pancreatic septa. Although one to three sections of this organ do not constitute adequate sampling, this limited type of evaluation revealed that more than half of our autopsy series had histologic evidence of "chronic" interstitial pancreatitis. There

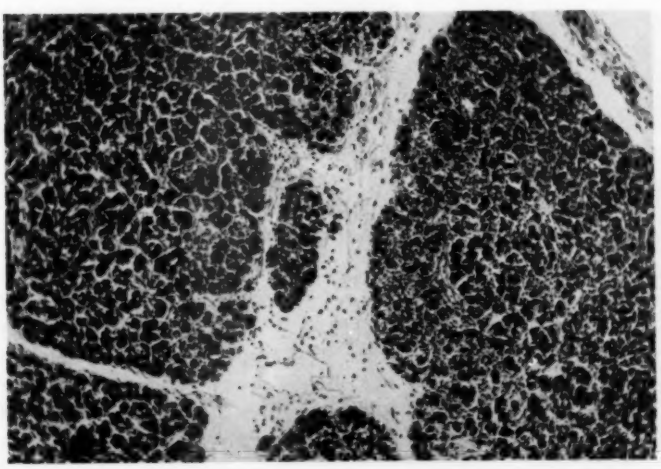

Fig. 1. — Widespread metastatic carcinoma. Focal pancreatic septal edema with mild infiltration by lymphocytes and few macrophages; reduced about 40% from mag. × 120.

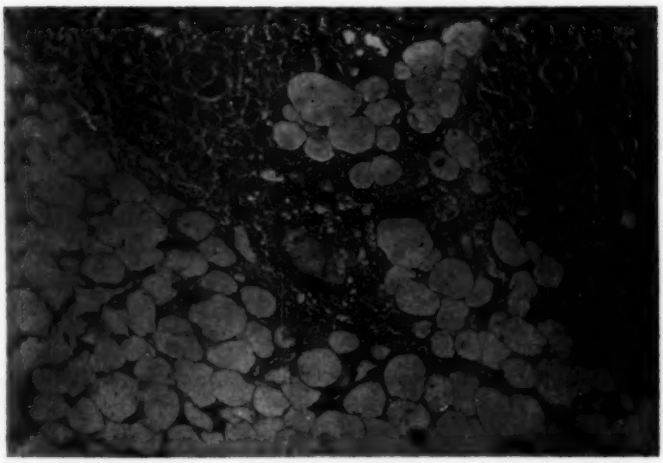

Fig. 2. — Disseminated malignant lymphoma. Focal fat necrosis with surrounding acute inflammatory-cell infiltration in pancreas; reduced about 40% from mag. × 120.

was no apparent association with the degree of vascular disease or the presence of squamous metaplasia of the duct epithelium. Grossly, the pancreas in each of these cases did not appear abnormal, and there was no suggestion of anatomical obstruction of the pancreatic duct. Furthermore, these patients had no recorded clinical or biochemical evidence of pancreatic dysfunction. However, in each case, review of the autopsy protocol revealed an obvious source of necrotic tissue precipitated by infection or neoplastic or abnormal vascular processes.

In this series, 7.8% of the cases showed histologic evidence of acute pancreatitis manifested by focal edema, neutrophilic infiltration, hemorrhage, and fat necrosis in varying degrees and combinations. However, gross alterations of this organ were recorded in the autopsy protocols in only three instances. None of these patients had had any clinical or supportive biochemical evidence of pancreatic disease. Although there was no apparent correlation with the degree of vascular disease, metaplastic changes in the duct epithelium, or any other local factor, each patient had a significant source of necrotic tissue precipitated by tumor, inflammation, or vascular disturbances.

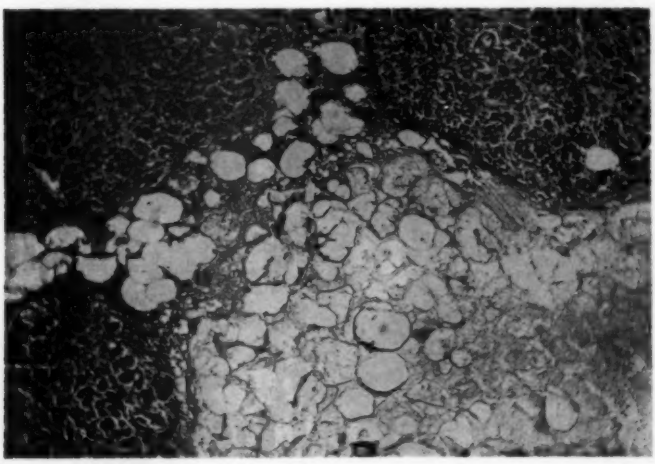

Fig. 3.—Acute peritonitis. Extreme fat necrosis with mild acute inflammatory-cell infiltration in pancreas; reduced about 40% from mag. × 120.

Experimentally, we have shown that the combination of pancreatic duct obstruction, anatomic or functional, and a source of active trypsin resulted in the development of acute hemorrhagic pancreatitis in dogs. After duct obstruction, active enzymes from local or distant sites may reach the interstitial tissues of the pancreas via several pathways, including ductointerstitial and venointerstitial mechanisms. These pathophysiologic pathways are similar to the pyelovenous and pyelointerstitial types of reflux mechanisms in the kidney which appear after obstruction of the ureter. We have observed that terminal pancreatitis of a local type occurs far more frequently than has been described or appreciated. Thus, to initiate acute pancreatitis, active enzymes may be brought to the pancreas from distant sites via the blood stream or normally secreted inactive enzymes may be activated locally or liberated locally after tissue destruction. In any event, the development of spontaneous pancreatitis begins in the interstitial tissues.

The nature of the inflammatory response and degree of anatomical disturbance is a result of the balance between the concentrations of active enzymes and antienzymatic mechanisms in the interstitial tissues of the pancreas.[2-6] Since proteolytic enzymes are liberated from all necrotic cells, it is apparent that many basically different types of disease processes, such as necrotizing inflammation, neoplastic degeneration, and ischemic or hemorrhagic necrosis of tissue, may result in pancreatitis when associated with mechanical or functional obstruction of the pancreatic duct. In addition, the debilitated state which accompanies any major illness may result in local reduction of antienzymatic activities, further facilitating the development of pancreatitis. We feel that this type of "chronic" interstitial pancreatitis may be a terminal event in the course of a disease process from which the patient may recover and which gives no clinical evidence of pancreatic disease. Subclinical acute interstitial pancreatitis, commonly focal in nature, is a terminal event which results from the accumulation of lytic enzymes in the interstitial tissues of the pancreas through ductointerstitial or venointerstitial reflux mechanisms.

Summary

Seventy-three per cent of 358 consecutive necropsies examined showed histologic evidence of interstitial pancreatitis. Since foci of edema and chronic inflammatory-cell infiltrate were found in the septa, 66% of these cases were histologically classed as "chronic" interstitial pancreatitis. Of these cases, 7.8% were considered as acute interstitial pancreatitis because of foci of septal edema, neutrophilic infiltration, hemorrhage, and acute fat necrosis. In all of these cases, the evidence of pancreatitis was interstitial and focal, and there were no associated clinical or biochemical evidences of pancreatic dysfunction. However, on rare occasions, the spontaneous development of classical anatomical acute hemorrhage pancreatitis, clinically unsuspected, may terminate some other major illness. All of our patients had some obvious source of necrotic tissue precipitated by malignancy, inflammation, the interruption of the blood supply to a part, or direct trauma. Necrotic tissue is a known source of trypsin and other active enzymes. These active enzymes may be transported via the circulation and reach the interstitial tissue of the pancreas by the venointerstitial reflux mechanism. Organic obstruction of the pancreatic duct was infrequent.

We feel that all such instances of pancreatitis, focal or diffuse, acute or "chronic," without clinical or biochemical evidence of dysfunction, are terminal events in the course of some other major illness. However, on occasion this complication may hasten the patient's death.

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Substrate Specific Phosphatases at pH 7.2 in Biliary Obstruction and Liver Cell Damage

Special Reference to the Bile Canaliculi in Obstructive Jaundice and Liver-Cell Necrosis

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With the Technical Assistance of Dolores Zaffuto

We have recently described the staining pattern of nonspecific phosphatase, 5-nucleotidase, and adenosine triphosphatase at pH 7.2 by the use of a modified lead phosphate technique.[1]

The adenosine triphosphatase stain, when used in frozen sections from material fixed in cold neutral formalin, permits an excellent and uniform demonstration of bile canaliculi in all species examined. It was applied in the present investigation to liver sections of animals under various experimental conditions and to human biopsy material from patients with obstructive jaundice and hepatitis. Sections were also stained for 5-nucleotidase, glycerophosphatase at pH 7.2, and glucose-6-phosphatase. Some of the observations made may lead to a better understanding of the pathogenesis of jaundice in biliary obstruction.

Material and Methods

Hepatic tissue was taken from freshly killed animals and from patients subjected to laparotomy. Frozen sections 8μ to 12μ in thickness were prepared from fresh tissue and from thin slices of liver fixed from 16 to 24 hours in a 6% solution of cold neutral formalin. Sections were incubated for periods varying from 5 to 120 minutes at 37 C. For the demonstration of adenosine triphosphatase (ATPase), the incubation mixture consisted of 20 ml. of a 125 mg. % solution of adenosine triphosphate (ATP) (obtained from Sigma Chemical Company, St. Louis), 20 ml. of 0.2 M tris (hydroxymethyl) aminomethane maleate (tris maleate) (pH 7.2) buffer, 3 ml. of 2% lead nitrate, 5 ml. of 0.10 M magnesium sulfate, and 2 ml. of distilled water.

For the demonstration of 5-nucleotidase an equivalent amount of muscle adenylic acid was used in the incubation mixture. For the demonstration of nonspecific phosphatase, the mixture consisted of 10 ml. of a 1.25% solution of sodium glycerophosphate, 5 ml. of the 0.2—tris maleate buffer, 20 ml. of 0.2% lead nitrate, and 5 ml. of distilled water. When slight cloudiness occurred, the mixture was filtered before use. Glucose-6-phosphatase was demonstrated by a modification of Chiquoine's technique.[2,3] After incubation, sections were washed in tap water and immersed in dilute ammonium sulfide for several minutes. The sites of enzymatic activity were thus revealed by deposits of black lead sulfide. Fresh-frozen sections were postfixed in neutral formalin for several hours. Sections were mounted on slides with glycerin, and the cover slips, rimmed with nail polish.

In addition, tissues were fixed routinely in 10% formalin and stained with hematoxylin and eosin and other staining procedures when indicated.

Experimental animals were albino rats of the Wistar strain and rabbits of mixed breeds. The following procedures were used:

1. Acute liver-cell damage in rats was induced by carbon tetrachloride or thioacetamide; 0.3 ml. of a 1:10 dilution of carbon tetrachloride in liquid petrolatum per 100 gm. of body weight was injected intraperitoneally, and animals were killed after 24, 48, and 72 hours. One per cent thioacetamide was added to a complete synthetic-rat-food diet containing 18% casein. Animals were likewise killed after 24, 48, and 72 hours.

2. Fatty livers without associated liver damage were produced in female rats by intraperitoneal injection of 1 mg. of DL-ethionine per gram of body weight on three successive days. Animals were killed 24 hours after the last injection.

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Department of Pathology, St. Catherine's Hospital.

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Fig. 1.—Photomicrograph of section from the liver of a normal rat, showing uniform staining of the bile canaliculi. Their lumina are slightly wider around a periportal field in the upper left half of the picture. Incubation time, 10 minutes; reduced 10% from mag. × 125. (All of the sections in Figures 1 to 9 were prepared from fresh tissues fixed overnight in neutral cold formalin for the demonstration of adenosine triphosphatase at pH 7.2. No counterstain was used. The sites of enzymatic activity are represented in black in the photomicrographs.)

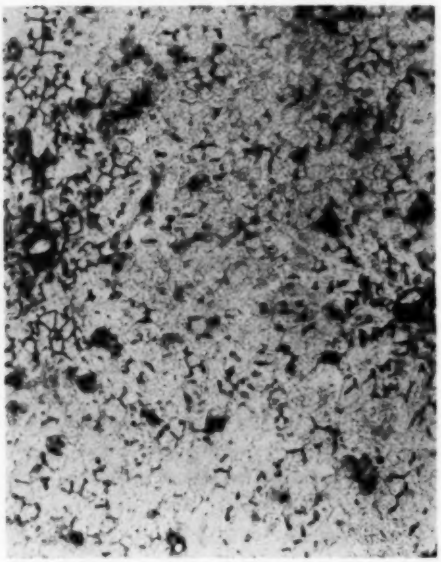


Fig. 2.—Photomicrograph of section from the liver of a rat killed 24 hours after the intraperitoneal administration of carbon tetrachloride. Only the bile canaliculi surrounding the periportal areas were stained. Incubation time, 10 minutes; reduced 10% from mag. × 125.

Fig. 3.—A higher magnification of the normal rat liver illustrated in Figure 1; reduced 10% from mag. × 350.

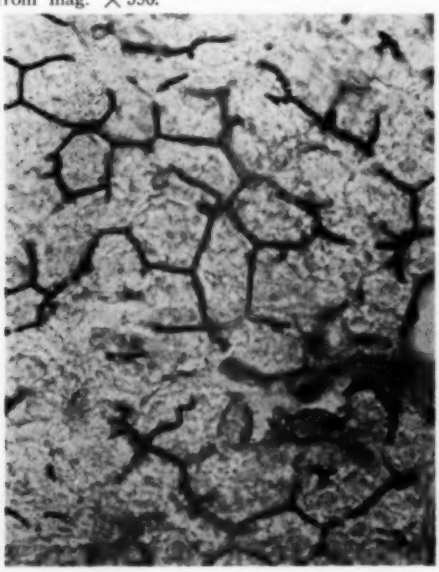


Fig. 4.—Photomicrograph of section from the liver of a rat killed four days after partial hepatectomy. There is striking increase in tortuosity of bile canaliculi, with the appearance of new intercellular branches and increase of round and rod-like structures in the vicinity of bile canaliculi. Incubation time, 10 minutes; reduced 10% from mag. × 350.

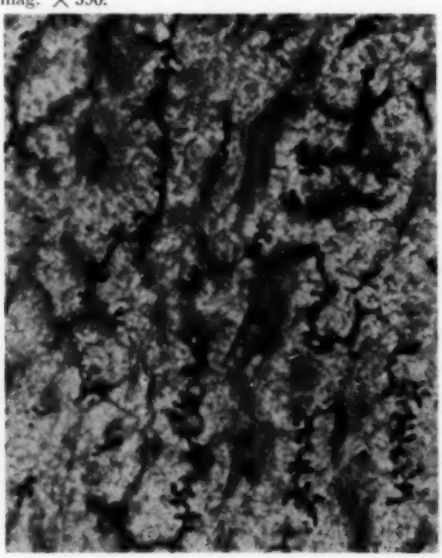


Fig. 5.—Photomicrograph of section from a normal rabbit liver, showing an area near a periportal field. Only bile canaliculi are stained. Incubation time, 5 minutes; reduced 10% from mag. × 350.

3. The influence of liver regeneration was examined in rats in which 60% to 70% of the liver was removed with use of light ether anesthesia. Animals were killed from one to seven days after the operative procedure.

4. The influence of bile duct ligation was studied in rats and rabbits. In the rat, the common bile duct was ligated with use of light ether anesthesia, and the animals were killed after 1 to 23 days. In the rabbits, the gallbladder was removed in addition. Animals were killed after 1 to 16 days.

Results

The normal distribution pattern of the various phosphatases in the three species was found to be as previously described.[1] In the case of adenosine triphosphatase, fresh-frozen sections were not as suitable as sections from tissues fixed in cold neutral formalin. Fresh sections revealed a varied degree of cytoplasmic staining and only irregular activity in biliary canaliculi. These structures, however, were stained regularly in fixed sections in all three species (Figs. 1, 3, 5, and 7).

In most instances, lead sulfide indicating enzymatic activity outlined a given canaliculus completely. Occasionally, it was depos-

Fig. 6.—Photomicrograph of section from the liver of a rabbit killed four days after ligation of the common bile duct. An area near a periportal field is shown. There is striking dilatation of the bile canaliculi, while sinusoids show almost no activity. Incubation time, 5 minutes; reduced 10% from mag. × 350.

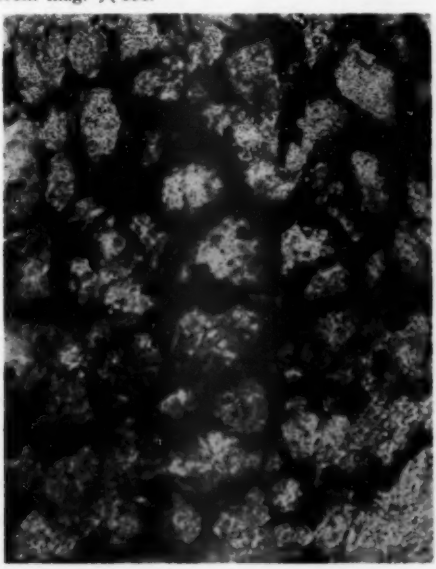
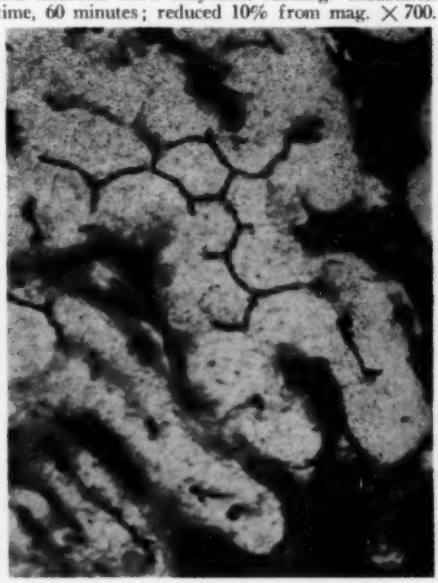


Fig. 7.—Photomicrograph of a biopsy specimen from a normal human liver. Biliary canaliculi and sinusoids show enzymatic staining. Incubation time, 60 minutes; reduced 10% from mag. × 700.



ited irregularly and gave the canaliculus a beaded appearance. The canaliculi were located between the liver-cell cords and formed the typical networks. Intercellular branches were numerous, and an occasional one extended close to a sinusoid. The canaliculi became wider in diameter as they approached the periportal fields. This was seen most distinctly in the rabbit. Biliary canaliculi did not always form straight lines. They sometimes appeared tortuous, with knob-like outpouchings. Positively reacting granules and rods were found close to the biliary capillaries in the rat and rabbit and only occasionally in man. The cytoplasm showed a weak staining reaction only after long incubation periods. Nuclei were not stained. The sinusoids gave a regular staining reaction in the human liver, reacted irregularly in the rat, and remained unstained in the rabbit. The central hepatic veins stained in the human liver. In the rat and human a diffuse reaction was also seen in periportal fields, walls of vessels, walls of bile ducts, and periportal connective tissue. The epithelium of large bile ducts did not react. In the rabbit, the reaction in the periportal fields was limited to the staining of some capillaries and the vascular endothelium.

In sections stained for 5-nucleotidase, the bile canaliculi were stained in the rat and man but not in the rabbit. In rat and man, but not in the rabbit, sinusoids stained very prominently, particularly in fresh-frozen sections. There was a positive reaction of connective tissue in rat and rabbit in periportal fields, while in man only the endothelium of blood vessels reacted positively.

Glucose-6-phosphatase was visualized only in parenchymal cells of the liver lobules in man and rabbit in a rather uniform pattern. In the rat, the periportal fields gave a stronger reaction than the central areas. When glycerophosphate was used as substrate, the distribution patterns were similar, although longer incubation periods were necessary. In man there was, in addition, staining of sinusoids.

1. *Experimentally Induced Liver-Cell Damage.*—A. Carbon Tetrachloride: Routine sections revealed the typical alterations, consisting of a varying degree of degenerative changes, with fatty infiltration, ballooning of cells, and frank necrosis in central areas within 24 hours. A moderate amount of inflammatory cells was also seen. In animals killed after 48 and 72 hours, remnants of necrotic cells were still present. There was a marked inflammatory reaction, with histocytic proliferation. Mitotic figures became very prominent in the parenchymal cells in the periportal areas. In preparations from animals killed after 24 hours and stained with the ATPase technique, diminution or complete loss of staining of bile canaliculi in necrotic cells was noticed (Fig. 2). This suppression of activity was often more extensive than was to be expected from sections stained with hematoxylin and eosin. Bile canaliculi in the cells of the periportal areas stained more prominently than in normal control sections. The suppression of enzymatic activity in central areas was also seen in sections stained for 5-nucleotidase, glycerophosphatase at pH 7.2, and glucose-6-phosphatase. In animals that were killed after two to three days, an increase in the staining reaction in the central areas was seen with the ATPase technique. Necrotic cells had a slightly darker hue than surrounding normal liver cells. Occasionally, bile canaliculi in necrotic cells as well as remaining sinusoids stained strongly. Increased staining reaction in central fields was often diffuse and could not be clearly localized. A similar, although often less marked, reaction was found in sections stained for 5-nucleotidase but not in those stained for glucose-6-phosphatase and glycerophosphatase. Bile canaliculi stained very prominently in the periportal fields. An increased amount of positively reacting granules was seen in their vicinity.

B. Thioacetamide: The changes noticed on routine sections were similar to those following the administration of carbon tetra-

chloride. Within 24 hours there occurred extensive necrosis of liver cells in central fields, accompanied by an acute inflammatory reaction. In the following days the necrotic cells gradually disappeared as histocytes and fibroblasts became prominent. In most instances the bile canaliculi in damaged liver cells in central fields did not react, and no staining was seen with the glycerophosphatase or glucose-6-phosphatase techniques. In several livers, however, necrotic cells showed a strong diffuse cytoplasmic staining reaction. This reaction was also seen in sections stained for alkaline phosphatase, even when the substrate was omitted from the incubation mixture. Moreover, control sections gave a positive reaction for calcium with the von Kossa stain.[3a] A similar nonspecific reaction for alkaline phosphatase due to calcium deposition has been previously found in necrotic liver cells following chloroform poisoning.[4] In animals killed after 48 to 72 hours, the central fields gave a strong, mostly diffuse, staining reaction comparable to that seen after carbon tetrachloride administration when either stained for ATPase or 5-nucleotidase. In the periportal nondamaged and regenerating areas bile canaliculi appeared somewhat dilated. More intercellular branches were apparent, and the liver cells contained an increased amount of granular material.

2. *Fatty Changes Without Associated Liver-Cell Necrosis.*—In the livers of female rats in which marked fatty changes had been induced by ethionine, bile canaliculi stained with either the adenosine triphosphatase or nucleotidase technique in a normal fashion. In the case of glucose-6-phosphatase and glycerophosphatase, lead deposits, indicating enzymatic activity, were often seen at the periphery of the cells, owing to the presence of the large fat droplets in the cytoplasm.

3. *Liver Regeneration.*—After the removal of 60% to 70% of the liver, only slight increase in sinusoidal staining was seen on the first day. In the livers of animals killed after two to five days, the bile capillaries appeared to be more tortuous. Their lumens appeared often wider than normal. There was also a marked increase in the number of intercellular branches. More granules and rod-like structures located in close proximity to the bile capillaries were also observed (Fig. 4). After five to seven days, these changes became less marked. No consistent difference in staining reactions for glucose-6-phosphatase and glycerophosphatase was found in these livers, although the central areas of the liver lobules often gave a stronger reaction than was seen in normal control livers.

4. *Experimental Biliary Obstruction.*—A. Rat Experiments: Routine sections revealed the typical changes described in detail by Cameron and Oakley.[5] Within 24 hours there occurred focal necrosis of the liver cells. In the following days fibrosis in periportal fields with proliferation of the bile ducts was seen. This fibrosis led within two to three weeks to considerable distortion of the liver architecture. In liver sections from animals killed 24 hours after ligation of the common bile ducts that were stained for ATPase activity only slight increase of granular deposits in the vicinity of the bile capillaries and some increase in the staining of the sinusoids was noticed. Necrotic cells reacted negatively with the various enzymatic staining reactions employed. After 48 hours the bile capillaries appeared slightly dilated and the amount of granules in the vicinity of the bile canaliculi was somewhat increased. In the following days, positive-reacting granules became still more numerous. The lumina of the bile capillaries became somewhat more dilated. The outline of the bile canaliculi was often blurred, and occasional intercellular branches abutted on the sinusoids. Sinusoidal staining was quite prominent. In animals killed in the second and third weeks after bile duct ligation, staining of sinusoids became very marked. Many of the bile canaliculi appeared moderately dilated, and an occasional one, markedly so. Other bile canaliculi showed, by that time, only thin lumina and could often barely be recognized. There was no block-

age by inspissated bile. Retained bile was seen in globular and granular form within the cytoplasm of some liver cells and gave a negative staining reaction. The bile duct epithelium did not react, but the periportal connective tissue reacted positively with both the ATPase and the 5-nucleotidase techniques. Glucose-6-phosphatase disappeared from areas in which liver cells had been replaced by proliferating bile ducts and connective tissue. Occasional liver cells in the vicinity of these areas gave a stronger reaction than surrounding liver cells.

B. Rabbit Experiments: Routine sections showed the typical changes.[6] Within 24 hours extensive areas of liver-cell necrosis were observed. Periportal fields showed increasing degrees of fibrosis and bile duct proliferation Within two weeks marked cirrhotic changes had developed. Sections stained with the ATPase technique revealed considerable dilatation of bile canaliculi within 48 hours. Often the outlines of the bile canaliculi were blurred. Granular material was not infrequently increased in the liver cells. Rod-shaped structures were noticed in large amounts in connection with the bile canaliculi. In the following days the dilatation of the bile canaliculi became still more marked. Granular and rod-shaped material was very prominent in many of the cells. Many intercellular branches abutted the sinusoids. The sinusoids, however, showed no or only a slight staining reaction (Fig. 6). In livers with marked cholangitic cirrhosis the bile canaliculi still gave a prominent, although less marked, reaction than was the case in the earlier phases. Areas of necrosis did not react. Within the periportal fields only capillaries and vascular endothelium were stained. With use of the technique for 5-nucleotidase, only the proliferating connective tissue in periportal fields gave a positive staining reaction. The areas of necrosis were negative in the preparation for glycerophosphatase and glucose-6-phosphatase. Occasional liver cells in the vicinity of proliferating periportal fibrous tissue gave a stronger staining reaction for glucose-6-phosphatase and glycerophosphatase as was seen in the rat livers with biliary obstruction.

Data on Patients from Whom Biopsy Specimens Were Taken

Case	Diagnosis	Duration of Jaundice, Days	Serum Bilirubin, Mg./100 Cc.	Alk. Phos'ase, K. A. Units
1	Obstructive jaundice due to stone	5	4.2	3
2	Obstructive jaundice due to stone	7	5.0	19
3	Obstructive jaundice due to stone	10	11.5	23
4	Obstructive jaundice due to stone	14	4.5	85
5	Obstructive jaundice due to stone	18	4.0	14
6	Obstructive jaundice due to stone	20	9.4	18
7	Obstructive jaundice due to ca of bile duct	25	11.5	23
8	Obstructive jaundice due to ca of pancreas	40	10.0	19
9	Subacute hepatitis	17	5.4	19.5
10	Subacute hepatitis	21	3.5	19.6

C. Human Material: Biopsy specimens of eight cases with obstructive jaundice were available for study. In addition, specimens of two livers of patients with subacute hepatitis could be used. The Table lists the causes of jaundice, its duration, and the preoperative values for bilirubin and alkaline phosphatase in the blood. In cases of obstructive jaundice, routine sections revealed the typical changes in the liver. After short duration of jaundice, retention of biliary pigment and bile thrombi were seen in central areas. In livers in which jaundice had been present for a longer period of time, such alterations were also present in the periphery of the liver lobules. In some sections, focal areas of liver-cell necrosis were found. The periportal fields showed a varying degree of fibrosis and infiltration by inflammatory cells. Changes in the bile canaliculi were noticed in all cases of obstructive jaundice. They could be demonstrated in sections stained both for 5-nucleotidase and for adenosine triphosphatase. Even in the liver sections from the two patients (Table, Cases 1 and 2) who had been jaundiced for only five to seven days, moderate dilatation of bile canaliculi was noticed (Fig. 8). The bile canaliculi appeared to be more tortuous, and positive-reacting rods and granules were quite

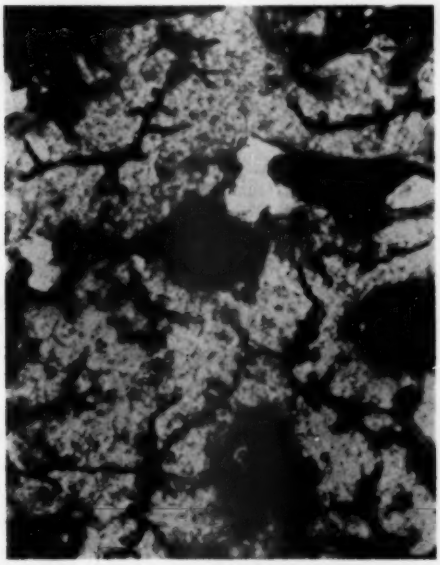


Fig. 8.—Photomicrograph of a biopsy specimen from the liver of a patient with a history of obstructive jaundice of seven days' duration (Table 1, Case 2). There is moderate dilatation of the bile canaliculi. Incubation time, 60 minutes; reduced 10% from mag. × 700.

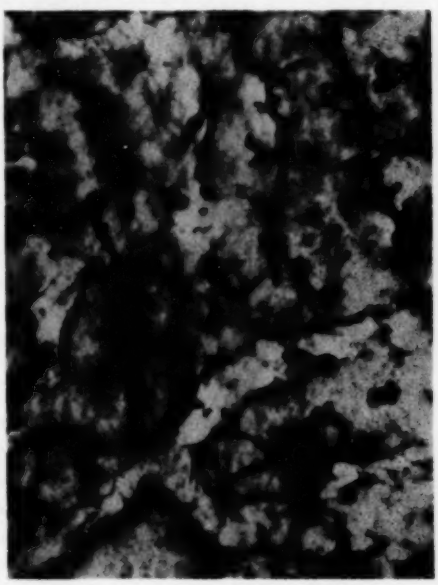
numerous in some liver cells. These structures were often in close proximity to the bile canaliculi and occasionally seemed to form a network in the cytoplasm. In sections of livers in which jaundice had lasted for longer periods of time, a more marked dilatation of the bile canaliculi was seen. Intercellular branches extended to the sinusoids (Fig. 9). The latter, however, showed no increased staining reaction.

Retention of bile pigment with the formation of bile thrombi often led to even greater distention of the bile canaliculi. The inspissated bile gave no or only a weakly positive staining reaction. Occasionally, the extrusion of bile thrombi into sinusoids was clearly recognizable. Wherever liver cells had become necrotic, the bile canaliculi failed to give a positive staining reaction. With the ATPase technique, a strong reaction was seen in periportal fields. On the other hand, only little activity was seen in this location in sections stained for 5-nucleotidase. It should be stressed that the changes in the bile canaliculi varied considerably in various livers.

With the technique for glycerophosphatase at pH 7.2 and glucose-6-phosphatase, necrotic cells did not react. Otherwise, the results with these two stains were no different from those in normal controls.

In the two cases of subacute hepatitis, the inflammatory reaction was seen mostly around periportal fields. In damaged liver cells, the bile canaliculi did not react. However, in the more central areas bile canaliculi stained often more intensely than in normal control livers. In sections stained for ATPase, there was very prominent activity in periportal fields, often obscuring the cellular details. It was, therefore, more advantageous to use the 5-nucleotidase technique, since the areas of inflammation did not react with this stain, permitting a clear delineation of the sinusoids and bile canaliculi.

Fig. 9.—Photomicrograph of a biopsy specimen from the liver of a patient with a history of obstructive jaundice of 25 days' duration (Table 1, Case 7). There is very marked dilatation of bile canaliculi. Intercellular branches extend to the sinusoids. Incubation time, 60 minutes; reduced 10% from mag. × 700.



Comment

The distribution patterns of the various phosphatases at pH 7.2 were similar to those described previously.[1] The technique for adenosine triphosphatase in all three species used in the present investigation and that for 5-nucleotidase in rat and man gave consistent and excellent visualization of the bile canaliculi. Bile canaliculi can also be stained with the technique for nonspecific alkaline phosphatase in the rabbit,[6-8] but the results with this technique are less uniform and reliable in the rat[9] and in man.[10-13]

In experimental hepatic necrosis induced either by carbon tetrachloride or by thioacetamide, the necrotic cells in the central areas showed no staining of bile canaliculi and the staining reaction was negative for glucose-6-phosphatase and nonspecific alkaline phosphatase at pH 7.2. Occasionally, a nonspecific cytoplasmic staining, obviously due to calcium deposition, was seen with all techniques after the ingestion of thioacetamide. After 48 and 72 hours the central areas gave a strong staining reaction with both techniques for 5-nucleotidase and adenosine triphosphatase but not for glucose-6-phosphatase. This staining reaction was often diffuse but seemed to be mostly localized in the remaining sinusoids and to less localized in necrotic cells. It seems probable that this staining reaction is due to diffusion and absorption of enzyme from the circulating blood, since considerable nucleotidase activity[14,15] and adenosine triphosphatase activity[15] can be demonstrated in the blood serum. Necrotic cells in livers with experimentally induced biliary obstruction and in human livers with obstructive jaundice reacted negatively to the various phosphatase stains. Degenerated cells in two cases of subacute hepatitis were also negative. Previously, it had been found that necrotic areas in the rabbit liver with obstructed bile ducts did not stain for alkaline phosphatase.[6] Necrotic liver cells after the administration of carbon tetrachloride were found to give either a negative,[16] a weak positive,[14] or a strong positive reaction[17] in preparations stained for alkaline phosphatase.

A marked increase in the staining of bile canaliculi after partial hepatectomy was found when either glycerophosphate[18] or muscle adenylic acid[19] was used as substrate at a pH of 9.0. The distention and stronger branching of biliary canaliculi was also demonstrated in preparations stained for adenosine triphosphatase. The proliferating undamaged cells in the periportal fields after the administration of carbon tetrachloride and thioacetamide also showed an increased staining reaction of the bile canaliculi. No consistent increase in either glucose-6-phosphatase or glycerophosphatase at pH 7.2 was found in livers following partial hepatectomy. This is in contrast to the pronounced increase in stainable acid phosphatase.[18]

The changes in the bile canaliculi after experimental biliary obstruction in the rabbit resembled closely those previously described when the technique for nonspecific alkaline phosphatase was used.[6,8] In general, dilatation of the bile canaliculi in the rabbit is strongest in the first week and somewhat less distinct in the following weeks. In the rat, a similar phenomenon was noticed. The distention of the bile canaliculi was, however, considerably less marked than in the rabbit. Although some bile canaliculi appeared to be dilated in animals killed after more than two weeks, other canaliculi stained even less prominently than was seen in normal livers.

In sections of the human livers with biliary obstruction, dilatation of the bile canaliculi was in general moderate although quite distinct even in the two instances in which jaundice had only been present for a period of four to seven days. Dilatation of intercellular branches with extension to the sinusoids was seen in all three species examined. Distention of bile canaliculi in human livers was most marked where inspissated bile had forced the adjacent liver-cell cords apart. Occasionally bile thrombi were seen in the process of extrusion into

the sinusoids. This phenomenon has been described many years ago by Ogata.[20]

Distinct increase in the staining of sinusoids with the adenosine triphosphate technique occurred in the rat but was only moderate in man and rabbit. In all three species, a very distinct increase of positive-reacting granules and rod-shaped structures occurred in the vicinity of bile canaliculi. Occasionally, these formed an intracellular network.

Kritzler and Beaubien [12] and Hard and Hawkins [8] studied livers with biliary obstruction with the technique for alkaline phosphatase and concluded that the increase in serum phosphatase commonly seen in biliary obstruction could be explained by the extension of the intercellular branches of bile canaliculi to the sinusoids, thus facilitating a direct transport of the enzyme from the bile into the blood. Observations with the adenosine triphosphatase technique in which very clear-staining pictures can be obtained support this contention.

In order to explain the regurgitation of bile into the blood stream, Eppinger [21] applied his stain for bile canaliculi to cases of obstructive jaundice and described not only dilatation but ruptures of these structures. However, Ohno [22] and Scheunert [23] could not confirm Eppinger's observations. Herring and Simpson [24] contended that the rapidity with which bile takes its normal course when the obstruction is removed would indicate that no significant mechanical rupture of the biliary system could have occurred. The results obtained by the application of the adenosine triphosphatase technique in conjunction with recent observations made with the electron microscope would support the opinion of Herring and Simpson.

Biliary canaliculi have no walls of their own but are in effect intercellular spaces bounded by the cell membranes of the adjacent hepatic cells.[25] According to Rouiller,[26] there are no definite limits to which the biliary canaliculi extend between the hepatic cells. The perisinusoidal spaces of Disse, which are interspersed between the liver cells and the sinusoids, communicate directly on one hand with the bile canaliculi and on the other hand with the sinusoids. The sinusoidal endothelium is not continuous, and there are large openings (20 to 200 $m\mu$ in size) within the walls of the sinusoids. In biliary obstruction, the bile canaliculi dilate, as can also be seen in electron micrographs, thus extending to the spaces of Disse. Such extensions of the bile canaliculi can be clearly recognized in preparations stained for ATPase activity. Communications of this kind have also been described by Pavel [27] in thin serial sections stained with Eppinger's method as well as with various routine techniques.

All of these findings suggest that a reflux of biliary constituents into the blood stream can occur without real tears in the bile canaliculi. The tendency of regression of the distention of bile canaliculi in longer lasting experimental obstructions may be due either to a widening of the submicroscopic openings between the Disse spaces and the sinusoids, thus lessening the areas of greatest resistance, or by a decrease in the excretory function of the liver.

Since bile canaliculi are often dilated and more prominent in the undamaged areas of livers in which liver-cell necrosis has occurred, it appears possible that the overloading of the bile canaliculi under these circumstances may be a contributory factor in the pathogenesis of hepatocellular jaundice.

Summary

The recently described adenosine triphosphatase technique, when applied to formalin-fixed sections, permits an excellent and uniform demonstration of the biliary canaliculi. It was used in the livers of rats in which acute central necrosis had been induced by the administration of carbon tetrachloride or thioacetamide. Sections were also stained for 5-nucleotidase, glycerophosphatase at pH 7.2, and glucose-6-phosphatase. Necrotic liver cells showed no staining of the

bile canaliculi and reacted negatively to the stains for glycerophosphatase and glucose-6-phosphatase in the first 24 hours. In the following days, however, the central areas showed a diffuse staining reaction with the adenosine triphosphatase stain, presumably due to diffusion of enzyme from the blood. In fatty livers produced by the administration of ethionine to female rats, bile canaliculi showed a normal staining pattern. Regenerating livers, after partial hepatectomy, as well as periportal areas in livers with central necrosis, revealed slight dilatation and increased staining of bile canaliculi.

In the livers of rats and rabbits with experimental biliary obstruction and in human biopsy material from patients with bile duct obstruction, dilatation of biliary canaliculi was regularly observed. It was most marked in the rabbit and of varying degree in man. In all three species intercellular canaliculi were seen to abut on sinusoids. In conjunction with recent electron microscopic findings, this observation indicates the possibility of a retrograde flow of biliary constituents into the blood stream without interruption of the anatomical integrity of the biliary system. It is also suggested that the dilatation of bile canaliculi in undamaged areas of livers with necrotic changes may indicate an insufficiency of the remaining bile canaliculi for the elimination of the biliary constituents and thus contribute to the occurrence of hepatocellular jaundice.

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Case Reports

Toxoplasmosis in an Adult, Presenting as a Space-Occupying Cerebral Lesion

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In the past decade, toxoplasmosis has emerged from a relatively obscure place in the small print of textbooks on tropical diseases to the status of a commoner disease. Increased interest has led to the development of many screening tests. Statistical studies utilizing these tests have reported that one-fourth of the general population has been infected at one time or another.[1] This growing familiarity with toxoplasmosis has led to a fairly well understood pathogenesis as lucidly expressed by Zuelzer [2] and Frenkel.[3] Toxoplasma gondii is an obligate intracellular protozoan organism.

Report of Case

A 36-year-old white woman was admitted to this hospital in September, 1955, with complaints of recurring severe occipital headaches which radiated to the frontal region. These were accompanied by insomnia, nausea, occasional blurring of vision, and scotomas. She had two children, both past adolescence, and had been a widow for over 10 years. The family history and other past medical history were noncontributory. Physical examination revealed an alert cooperative woman who had bilateral papilledema, weakness of the left arm and left side of the face, and an increased left knee reflex. There was no sensory loss. The Babinski reflex was absent. The remainder of the physical examination was not remarkable. Blood pressure was 145/85. The pulse was regular at 74 beats per minute. Serum VDRL test was negative. The spinal fluid showed 780 erythrocytes, 2+ globulin, and 18 white cells, with a differential count of 12 polymorphonuclear leukocytes and 6 lymphocytes. Spinal fluid cultures for predominating organisms, tuberculosis, and fungus infection were negative. Skull x-rays and bilateral arteriograms were negative. However, the EEG was thought to suggest a right frontal focus. The ventriculogram indicated a large deep right frontal space-occupying lesion.

Surgery was performed, with partial excision of a "tumor" deep in the right frontal lobe. Frozen section was reported as "astrocytoma, grade I or II." The surgically removed "tumor" consisted of an irregular gray avascular mass of soft tissue which imperceptibly faded into the adjacent normal cerebral tissue. Throughout the brain tissue there were scattered petechiae and a moderate amount of edema.

Microscopic Findings (Figs. 1, 2, 3, and 4).—The lesion consisted of a vascular granulomatous inflammation containing lymphocytes, plasma cells, and multinucleated giant cells bordering on necrotic tissue. Occasional "miliary" lesions were seen. There were scattered cyst bodies, having definite capsules and ranging up to approximately 50μ in diameter. These contained packed clusters of discrete periodic acid-Schiff (P. A. S.) -positive organisms. The vessels showed moderate hypertrophy of the endothelium, with perivascular collections of mononuclear cells. The final microscopic diagnosis was "chronic granulomatous inflammation containing organisms resembling Toxoplasma."

Methyline blue dye test performed at the Federal Public Health Hospital in Chamblee, Ga., was re-

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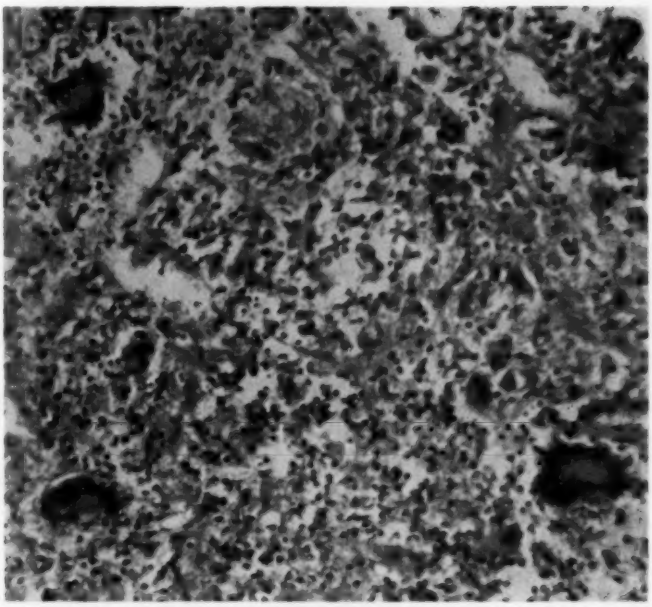

Fig. 1.—Severe inflammation of brain, with scattered multinucleated giant cells; × 338.

ported on Oct. 28, 1955, as positive, with a titer of 1:256.

Postoperatively, the patient developed left hemiparesis but was discharged at the end of October as showing fair recovery.

She was readmitted in December, 1955, because of persistent left hemiparesis and complaints of poor visual acuity, diplopia, nausea, and frequent right temporofrontal headaches. Skull x-rays showed no evidence of any destructive process. EEG had the same abnormal focus in the right frontotemporal region.

The dye Toxoplasma test was repeated and reported on Dec. 14, 1955, as positive, the titer

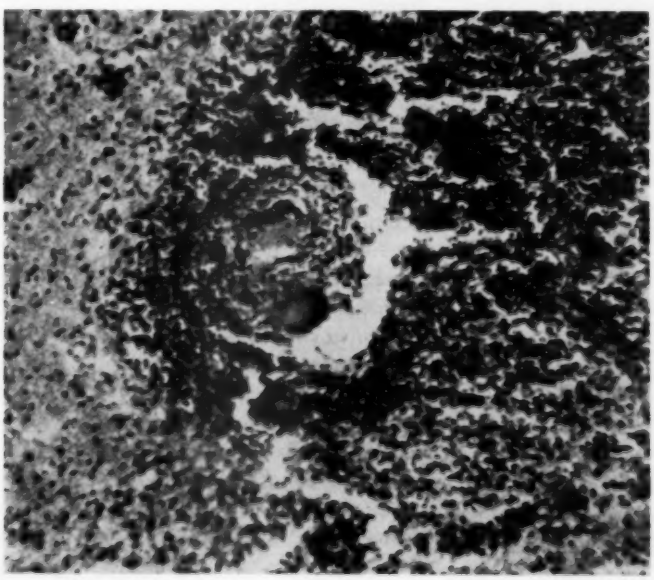

Fig. 2.—"Miliary" granulomatous lesion; × 300.

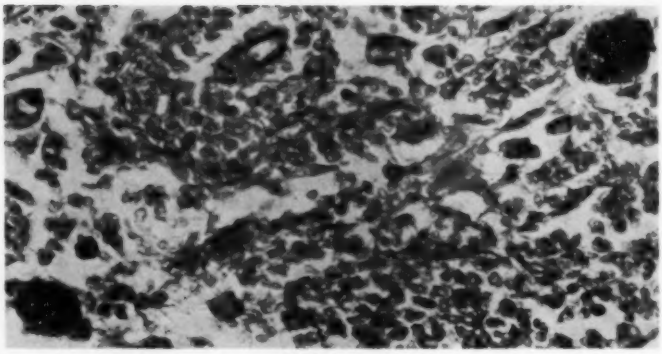

Fig. 3.—Two encapsulated cyst bodies containing Toxoplasma organism. Periodic acid-Schiff; reduced 25% from mag. × 477.

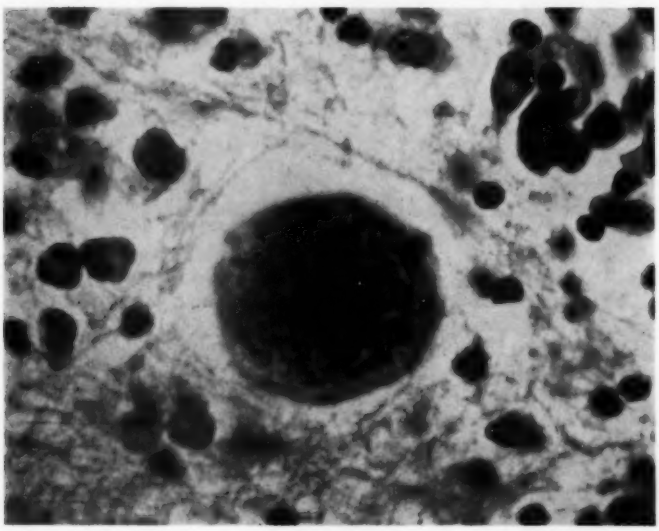

Fig. 4.—Cyst body containing Toxoplasma organisms. Hematoxylin and eosin; × 783.

being 1:1024. The patient was treated with sulfadiazine and pyrimethamine without noticeable improvement. Although the prognosis for real improvement of the neurological defects was poor, physical therapy did improve her gait and allow some rehabilitation.

Comment

Pinkerton and Weinman [4] believe that Toxoplasma organisms were first seen by Laveran, in 1900, in Java sparrows, and credit Castellani with first suggesting the possibility of human infection, in 1913 and 1914. In the succeeding period of about 40 years, reports of the disease have steadily increased in frequency. Today it is possible to read of the diagnosis of toxoplasmosis in patients with only vague constitutional symptoms, as described by Syverton and Slavin,[5] or with only lymphadenopathy, as in Siim's [6] report of seven cases. However, a review of the literature listed in the "Quarterly Cumulative Index Medicus" from 1926 to 1956 fails to disclose a single report of toxoplasmosis in an adult in which the presenting signs and symptoms were referable to the central nervous system. In a personal communication, Dr. J. K. Frenkel states that he has no knowledge of a similar case.[7] Kass et al.[8] have reported a case of this infection in an adult in which the presenting complaint was of the nature of "spotted fever." This patient did manifest

mental disorders a few weeks after admission, and at necropsy there were pseudocysts in the myocardium, striated muscle, and nervous system. Callahan et al.,[9] in their review, discovered that some of the pediatric deaths signed out as chronic meningitis were in reality due to cerebral toxoplasmosis. Pediatricians have been impressed with the presenting symptom of convulsions (Cowen et al.,[10] Crothers,[11] and Wolf et al.[12]). Ophthalmologists have emphasized the sign of chorioretinitis (Wagener,[13] Wilder,[14] and Vail et al.[15]). The resemblance of this protozoan to Leishmania, as discussed by Jacobs,[16] has given it a more significant position among the tropical diseases. In spite of this probable relationship to the tropical protozoan infections, one of the latest editorials [17] states that Toxoplasma is worldwide in distribution and is pathogenic for all Vertebrata. The entire subject of neonatal toxoplasmosis has been well summarized (Frenkel and Friedlander [18] and Frenkel [19]).

Currently, cerebral toxoplasmosis in children is well known as an encephalomalacia caused by granulomatous lesions, frequently calcified, scattered in the brain. Chorioretinitis is an important accompaniment in children. In adults the disease has been described only as a generalized viral-like infection, with lymphadenopathy and interstitial pneumonitis or with a generalized skin rash (Pinkerton and Henderson [20] and Kass et al.[8]). Diagnosis is made either on serum studies or microscopic examination of postmortem material (Alexander and Callister,[21] Jacobs,[22] and Brown and Jacobs [23]). Frenkel [19] has stressed the emergence of immunity and a delayed type of hypersensitivity in both men and animals, with development of lesions during partial immunity in mice and hamsters.

In our case the only proven lesion was in the frontal lobe of the cerebrum, which was removed surgically. No clinical evidence pointed to other foci of infection.

The epidemiology of the disease is still in doubt. Plaut [24] states the almost definite probability of human carriers. Callahan [25] found that 2.7% of tested persons in the St. Louis area showed definite inhibitory factors in their serum against the necrotizing action of Toxoplasma in the skin of rabbits. Jacobs [16] has suggested that the arthropod vector is the most likely source of infection, because Toxoplasma gondii is thermolabile and requires an intracellular environment for survival. Both these conditions are adequately furnished by an arthropod. Most recently, Weinman and Chandler [26] have proposed an intriguing mechanism of transmission, namely, through the consumption of undercooked pork. This presents the most plausible theory for the origin of the adult infection, which is then transmitted to the infant through the placenta (Beckett and Flynn [27]). Our patient lived in Pennsylvania all her life, except for a short trip to Kentucky in 1947. Neither of these states is particularly noted for arthropod infestation. Moreover, there is no history of corticoid therapy, and so resumption of a latent infection as stressed by Frenkel [19] seems unlikely. There is no evidence that her two children are infected. Other contaminated vehicles, such as water, milk, or farm produce, might be at fault.

Preventive measures, the ultimate goal in disease, are at present out of the question, although more evidence for the incrimination of swine would change the outlook. Consequently, the challenge falls to therapeutics. Eyles and Coleman [28] and Wettingfeld et al.[29] have shown that the best regimen available today is sulfadiazine and pyrimethamine. The patient of this report received these drugs in the prescribed doses, as well as preoperative complete therapeutic trials of most of the other broad-spectrum antibiotics.

Summary

A case of cerebral toxoplasmosis has been described, occurring in an adult and presenting as a space-occupying intracranial lesion. An excised granulomatous lesion was diagnosed as toxoplasmosis by histologic and serologic means. The literature is briefly summarized. To our knowledge no adult

case has previously been reported presenting with cerebral signs and symptoms.

Dr. Stuart Rowe gave permission for the use of the case; Dr. J. K. Frenkel provided interest and aid in this study; Dr. M. M. Brooke performed the Toxoplasma dye tests and gave advice.

Institute of Pathology, Western Pennsylvania Hospital (Dr. Reed).

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News and Comment

ANNOUNCEMENTS

Medical Research Foundation for Tay-Sachs' Disease and Allied Disorders.—A new nonprofit foundation has been established to support and stimulate research, clinical, and educational programs in Tay-Sachs' disease and allied heredofamilial neurodegenerative diseases of infancy and childhood. The scope of the program will include, in addition to Tay-Sachs' disease, Niemann-Pick's disease, infantile Gaucher's disease, Schilder's disease, diffuse sclerosis, amyotonia congenita, Friedreich's ataxia, and others. The foundation is known as National Tay-Sachs Association, Inc., New York Chapter, and is composed of parents who have had afflicted children, interested laymen, and medical personnel in the field.

The foundation is cooperating with the existing clinical and research program on Tay-Sachs' disease and certain of these allied diseases at Jewish Chronic Disease Hospital in Brooklyn. The hospital is presently conducting a special clinic for outpatient care of afflicted children and is constructing a special ward for the care and observation of inpatient cases. Both of these programs are being conducted in conjunction with the laboratory research program of the Isaac Albert Research Institute of the hospital. A comprehensive genetic study is also being made of the pertinent hereditary patterns from histories supplied by the foundation and parents who have children under the care of the hospital. A counseling program is also offered.

The operation of the foundation is on a nationwide basis, and contact is being made for establishment of chapters in other metropolitan areas, in addition to the allied Tay-Sachs Association in Philadelphia. In order to further its work and, in particular, to prepare a substantial genetic study covering the entire country, physicians and hospitals are requested to make the existence of the foundation known to parents of children afflicted with these diseases.

For further information on the foundation and its work, write to Medical Committee, National Tay-Sachs Association, Inc., New York Chapter, P. O. Box 1250, G. P. O., New York 1.

Index to Journal of the National Cancer Institute.—The National Cancer Institute has just issued a cumulative index of the *Journal of the National Cancer Institute* for the period 1940-1956. Included are a subject index, with comprehensive cross references; an author index, and a listing of current nomenclature for certain chemical compounds mentioned in the index. This should be a valuable addition to those libraries which have files of the *Journal,* as well as to research scientists in general, since it gives a rather comprehensive view of a large segment of research in the field of cancer during the 16-year period.

Copies of the index have been sent to all libraries and persons now receiving the *Journal.* A limited number of free copies are available. Single copies may be requested from the National Cancer Institute, Attn.: Information Officer, Bethesda 14, Md.

The National Cancer Institute has a few copies of some previous issues of the *Journal* and will fill requests for these issues from scientific libraries and investigators as long as the supply lasts.

PERSONAL

Appointment for Dr. Arthur C. Allen.—Dr. Arthur C. Allen has been appointed Professor of Pathology at the University of Miami School of Medicine and attending pathologist at the Jackson Memorial Hospital.

Appointment for Dr. Gordon R. Hennigar.—Dr. Gordon R. Hennigar has been appointed Professor of Pathology at the State University of New York College of Medicine, Downstate Medical Center, Brooklyn. He has also been appointed Chief Pathologist and Associate Director, Institute of Pathology, Kings County Hospital of Brooklyn. Dr. Hennigar was formerly Associate Professor of Pathology in the Medical College of Virginia.

Appointment for Dr. Robert W. Wissler.—Dr. Robert W. Wissler has been named chairman of the Department of Pathology in the University of Chicago, effective Oct. 1. He succeeds Dr. Paul R. Cannon, who has retired, but who will continue as Chief Editor of the A. M. A. ARCHIVES OF PATHOLOGY.

Appointment for Dr. Lewis Thomas.—Dr. Lewis Thomas, professor and chairman of the Department of Pathology in the New York University College of Medicine, has been named professor and chairman of the Department of Medicine, this appointment to become effective at the close of the 1957-1958 academic year.

Appointment for Dr. Joseph Imbriglia.—Dr. Joseph Imbriglia has been appointed as head of the department of pathology at Hahnemann Medical College and Hospital of Philadelphia.

Dr. Frank Forry Becomes Professor Emeritus.—Dr. Frank Forry, Professor of Pathology at the University of Indiana School of Medicine for many years, has become Professor Emeritus. Dr. Forry continues to carry on work in the Department of Pathology.

Dr. John C. Bugher Presents Lecture.—Dr. John C. Bugher, Director of Medical Education and Public Health of the Rockefeller Foundation in New York, presented the Second Annual Carl V. Weller Lecture, Dec. 14, in the University of Michigan's Horace H. Rackham Amphitheater. Dr. Bugher's topic was "The Role of the Pathologist in Medicine."

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Books

Progress in Gynecology. Volume III. Edited by Joe V. Meigs, M.D., and Somers H. Sturgis, M.D. Price, $15.50. Pp. 780, with Illustrations. Grune & Stratton, Inc., 381 Fourth Ave., New York 16, 1957.

This is the third volume in a distinguished series dealing with gynecologic progress.

The major advances which have occurred during the past five years in this field have been covered by a series of expertly chosen articles which are written by persons who are authorities in their respective fields. The subject matter ranges from pelvic vascular surgery to functional disorders of the female reproductive tract. The inclusion of papers on carcinoma in situ, the cytologic prognosis in cervical malignancy, cytologic tests for chromosal sex, the genetic effect of irradiation, the legal aspects of artificial insemination, and the use of radioactive colloidal gold for the treatment of cervical and ovarian malignancy are timely. Sections are devoted to recent advances in the management of infertility in the male as well as in the female, the treatment of female reproductive tract malignancy, and preoperative and postoperative care.

The material contained in this work supplements that of the preceding two editions, with the result that these three volumes constitute a comprehensive survey of current gynecologic concepts.

The present volume, as well as its predecessors, are valuable contributions to medical literature.

Perinatal Loss in Modern Obstetrics. By Robert E. L. Nesbitt Jr., M.D. Price, $12.50. Pp. 432, with 108 illustrations, including 10 color. F. A. Davis, Company, 1914-1916 Cherry St., Philadelphia 3, 1957.

This book on perinatal loss in modern obstetrics is a contribution to a field in which interest has increased considerably in the last few years. Until recently all efforts were directed toward preventing fatal complications in the mother, but the safety of pregnancy has now so greatly increased that more attention can be paid to the fetus. In the present work the conditions leading to unsuccessful pregnancy are summarized and suggestions as to possible prevention are given.

The book begins with a survey of the problem of perinatal mortality and then discusses the general problems of abortions, prematurity, and intermediate and late fetal deaths. The main pathologic disturbances are described in separate chapters; included are anoxia, birth injury, malformations, infections, and hemolytic disease. Multiple gestation, ectopic gestation, analgesia and anesthesia, and factors in the prevention of perinatal loss are the concluding chapters.

The clinical aspects of the problem are handled adequately, and the pathologic aspects, although brief and somewhat superficial, are sufficient for the clinician. Except for a few photomicrographs the illustrations are of regretfully poor quality.

The Collected Papers of Paul Ehrlich. Volume 1. Compiled and edited by F. Himmelweit, under the editorial direction of Sir Henry Dale. Price, $85 for 4 volumes. Pp. 653, with 6 plates. Pergamon Press, Ltd., 4 & 5 Fitzroy Sq., London W 1, 1956.

The first volume of Ehrlich's collected papers contains his work up to 1890, being mainly on anilin dyes as hematological and bacteriological stains and histochemical reagents and in general on the application of chemical methods to the study of physiological and pathological problems. Later volumes will be devoted to his work on immunology and cancer (Volume II), chemotherapy (Volume III), and the bibliography with indexes (Volume IV).

The papers were edited by Sir Henry H. Dale, who wrote an introduction in the first volume in which he gives an excellent evaluation of Ehrlich and publishes a letter in which Ehrlich himself analyzed the nature and sources of his scientific ideas and his methods of research.

The first volume contains several hitherto unpublished papers, such as Ehrlich's first work concerned with the discovery of the mast cells and his M.D. thesis, which presents Ehrlich's basic ideas on the relationship of chemicals to body constituents and living organisms. These

ideas are traceable throughout his subsequent research, and in this volume they are illustrated by his discoveries of granulocytes, reticulocytes, acid-fastness, diazo and azomethin reactions, dehydrogenation in biological oxidation, and the application of the biochemical affinities of dyes to therapy. These and other observations proved to be embryos developing into distinct disciplines in our times.

Although most papers are printed in the original language, the important papers, such as "The Requirement of the Organism for Oxygen," "Contributions to the Theory and Practice of Histological Staining," and "The Relationship Existing Between the Chemical Constitution, Distribution and Pharmacological Action," are translated in English as well. This should greatly help students of biological sciences to penetrate the sometimes complicated German scientific language.

Ehrlich's experience taught him that success followed upon long discouragement. In this lies one of the great educational values of his collected papers.

The Story of Peptic Ulcer. By Richard D. Tonkin, M.D., F.R.C.P., characterized by Raymond Keith Hellier, F.R.S.A. Price, $2.25. Pp. 71. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1957.

In relating the story of peptic ulcer, caricatures and terse comments are nicely combined to give the dyspeptic a short, amusing, but thoroughly factual insight into his disease.

Clinical Use of Radioisotopes. By William H. Beierwaltes, M.D.; Philip C. Johnson, M.D., and Arthur J. Solari, B.S., M.S. Price $11.50. Pp. 456, with 125 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1957.

This very excellent book is filled with practical useful information concerning the clinical application of radioisotopes. It has a readable style, with clear descriptions, good illustrative examples, and exhaustive references. The main body of material concerns radioactive iodine, (I^{131}). Clinical uses of I^{128}, I^{131} thyroidectomy, and I^{131} in the diagnosis and treatment of thyroid neoplasms are well discussed. Other chapters cover radioactive gold, Au^{198}; phosphorus, P^{32}; chromium, Cr^{51}; cobalt, Co^{60}; iron, Fe^{59}, and other less commonly used isotopes. Included are the rationale, technique, and calculations for frequently used diagnostic tests, e. g., I^{131}HSA and Cr^{51} blood-volume determinations. The therapeutic uses are succinctly outlined. Two of the outstanding features are a section on the recommendations and requirements of the AEC for those who wish to use radioisotopes in clinical medicine and a chapter outlining the guiding principles on starting and managing a clinical radioisotope unit.

Alcoholism, Basic Aspects and Treatment. By Harold E. Himwich. Price, $5.75. Pp. 220, with 33 illustrations. American Association for the Advancement of Science, Washington, D. C., 1957.

A presentation of the most recent advances in the basic physiological effects of alcohol and in the treatment of alcoholism is to be found in the papers of this symposium. A few of the interesting papers in the first half of this book deal with the following considerations: thyroid administration in rats reduces their appetite for alcohol; impaired carbohydrate utilization of Wernickes' syndrome persists despite clinical improvement on thiamine; thiamine-deficient rats show a normal adrenal response after alcohol injection, while deficiency of other B-vitamins prevents the normal adrenal response to alcohol. In the second half of this book the treatment of acute alcoholsm, delirium tremens, and the social problems created by alcoholism are considered. A major feature of this section is the appraisal of many new drugs in the therapy of alcoholism.

Anyone who deals with alcoholism would appreciate this book and the succinct summaries at the end of each paper.

Urological Surgery. By Dr. Austin Ingram Dodson. Price, $20. Pp. 868, with 664 illustrations. The C. V. Mosby Company, 3207 Washington Blvd., St. Louis 3, 1957.

The problems that confront the urologic surgeon are considered in great detail in this book. While physiologically sound surgical approaches to virtually every type of urological problem known constitute the main subject matter, new sections on the adrenal glands, urinary tract dysfunction due to nervous system disorders, and ureterosigmoidostomy, as well as further considerations of postoperative case and endocrine therapy of the prostate, materially add to the value of the third edition of Dr. Dodson's well-known book. The surgical pathologist as well as the urologist could profitably use this book.

Histologischer Atlas gynäkologischer Erkrankungen. By Dr. med. Herbert Lax. Price, DM 92. Pp. 199, with 226 illustrations. VEB Georg Thieme, Hainstr. 17/19 Aufg. C, Leipzig C. 1, 1957.

Herbert Lax, a disciple of Robert Meyer and Robert Schroeder, is one of the most outstanding gynecological pathologists as well as an outstanding research worker and clinical gynecologist. His present histological atlas of gynecologic diseases represents an expression of his own great experience and the teachings of R. Meyer and R. Schroeder. The atlas contains 226 most excellent colored illustrations. The chapters discuss the diseases of the vulva, vagina, and uterus and treat extensively the physiological and pathological endometrial changes and the diseases of the Fallopian tubes and ovaries. His opinions regarding diagnosis and clinical classification of early cervical carcinomas deserve special mention.

The monograph is vividly written and is not only of interest to the pathologist but also of importance to the clinical gynecologist. It should be seriously considered for translation into the English language.

Spontaneous and Habitual Abortion. By Carl T. Javert, M.D. Price, $11. Pp. 450, with 196 illustrations. McGraw-Hill Book Company, Inc., 330 W. 42d St., New York 36, 1957.

Papers concerned with various aspects of abortion are frequently found in the medical literature, but an extensive review of the subject has not been published for many years. Consequently, this work, which is based on a study of two thousand cases observed at the New York Lying-In Hospital between June 1, 1947, and June 1, 1954, and on an intimate acquaintance with most of the papers that have been written on this subject, is very welcome. In order to obtain material for this treatise, the author has participated in the examination of the aborted material as well as in the clinical investigation and treatment of the patients. A description of every specimen and data obtained by questioning and examining each patient have been carefully recorded and put on IBM cards. The data thus obtained form the basis for the discussion.

The first half of the book consists principally of an analysis of the gross and microscopic appearance of the aborted material. The author stresses the importance of cord anomalies, including twisting, as a cause of death, a view with which many able investigators disagree. He also found many abnormalities in decidua and placenta. The proportionately low incidence of actual malformations led him to conclude that environmental disturbances greatly outweigh genetic factors as a cause of abortion. The second half of the book is concerned with the clinical aspects of abortions and the general characteristics of the women from whom the specimens were obtained. The author has developed very definite ideas about how abortions may be prevented and devotes many pages to their discussion. The three main aspects of the problem he designates as antepartum bleeding, uterine contractions, and psychodynamic factors. To prevent these he stresses preparation for pregnancy, both physical and emotional; establishment of good nutrition aided by vitamin C, P, and K supplements, and active psychosomatic therapy. The relative importance which the author attaches to the psychic aspects of abortion are illustrated in a drawing showing the ten steps to a successful pregnancy. Two of the steps are high citrus diet and vitamins C, P, and K; the other eight are faith, love, psychosomatics, early prenatal therapy, no fears, security, teamwork, happiness.

The book contains a large amount of valuable material which will be very generally appreciated. Many readers, however, will regret the attempt of the author to be "folksy" in his style and will find the drawings illustrating the second half of the book in strange contrast with the excellent illustrations of the first half. Perhaps the statement of the author in the preface that he hopes the book will be useful to husbands, friends, relatives, clergymen, and so on, as well as to doctors, explains many things. It is difficult to write a book for a universal audience.

Lupus Nephritis. By Robert C. Muehrcke, M.S., M.D.; Robert M. Kark, F.R.C.P., F.A.C.P.; Conrad L. Pirani, M.D., and Victor E. Pollack, M.B., M.R.C.P.E. Price, $3. Pp. 145, with 43 illustrations. Williams & Wilkins Company, Mount Royal and Guilford Aves., Baltimore 2, 1957.

The authors present a thorough systematic review of an aspect of chronic renal disease which is being recognized with increasing frequency. Where in the past most long-standing renal disease was assumed to be chronic glomerulonephritis, careful consideration of large series

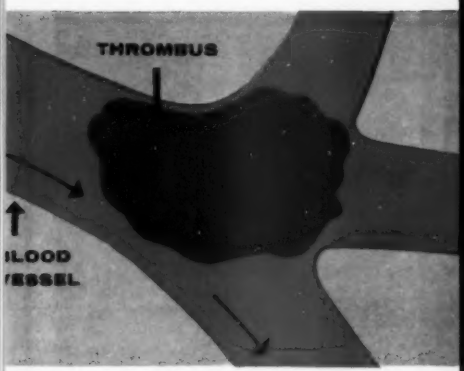
now reveals many cases of systemic lupus. The intensive use of modern therapy in this condition with prolongation of the course has increased the incidence and significance of the renal involvement. In this book the changing clinical status and laboratory findings are correlated with the serial pathologic changes followed by renal biopsies to present the natural history of lupus nephritis.

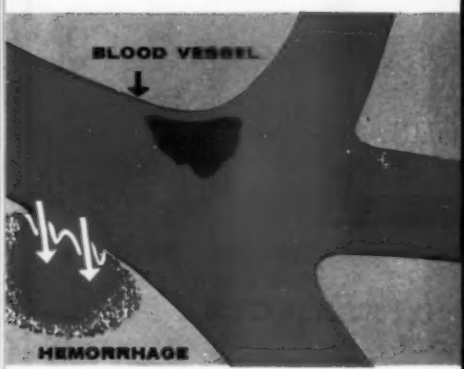
Separate chapters consider clinical features, renal pathology, etiology and pathogenesis, methods and results of this study, clinicopathologic features, treatment, prognosis, and the relationship to other diseases. References are recent and comprehensive. The text is well illustrated, with some photomicrographs in color. It will be helpful to those interested in both understanding and diagnosing chronic renal disorders.

A Manual of Pharmacology and Its Applications to Therapeutics and Toxicology. Eighth Edition. By Torald Sollmann, M.D. Price, $20. Pp. 1535. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1957.

Almost ten years have passed since the last edition of Dr. Sollmann's "Manual of Pharmacology" appeared. During this time rapid advancements have been made in almost every field of pharmacology, and because of the encylopedic manner in which each subject has always been handled in this book, its revision had been eagerly anticipated. As in previous editions, the book is designed for use as a text in medical school pharmacology courses by placing the essential material in large type. Research workers in the medical sciences will probably derive the greatest benefit from the wealth of material contained in the small type and from the extensive bibliography (over ten thousand references). Since the bibliography is restricted to publications appearing since Jan. 1, 1940, it is necessary to consult previous editions for references to the older literature. The arrangement of the book is similar to that of previous editions, with the addition of several new chapters dealing with such groups as the psychopharmacologic drugs, antituberculosis drugs, radioactive drugs, and the newer antibiotics and antimalarials. The information on a few of the older drugs has been condensed or deleted, but most sections appear to have been rewritten and expanded, so that with the addition of the new material, the size of the book has been increased by some four hundred pages. Dr. Sollmann has retained the practice of critically evaluating the status of the drugs in use in current therapy, which has always been one of the most valuable aspects of his book. This comprehensive treatment extends to drugs introduced as recently as 1955. The book is very readable, although concisely written, and the typography and reproduction of the relatively few figures is good.

Although it is rather expensive for use as a text, few will contest its value as one of our major references in pharmacology and experimental therapeutics. It is, indeed, inspiring to find that, in spite of his advancing years, Dr. Sollmann has produced a book which equals or surpasses the high standard established by previous editions.





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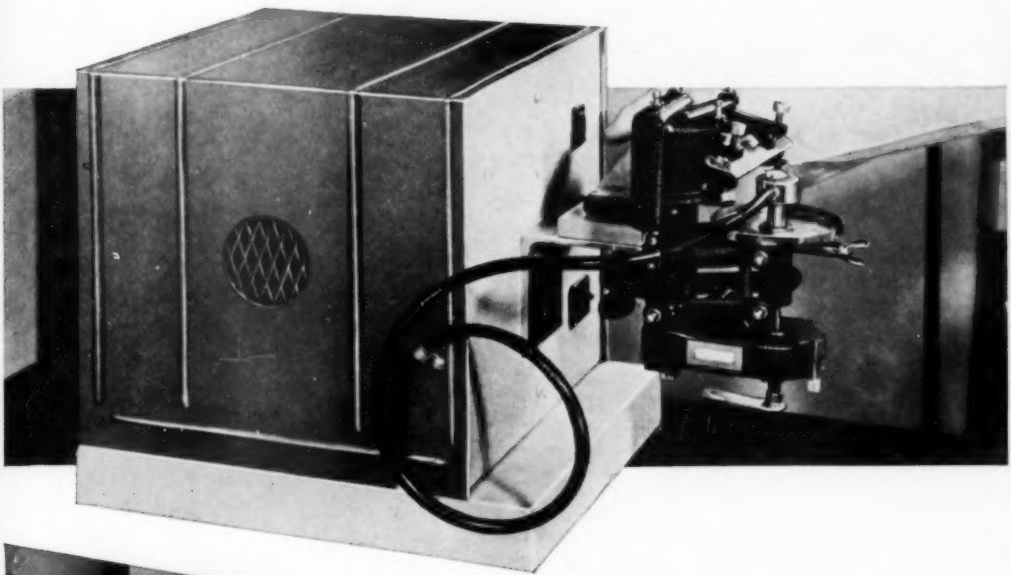
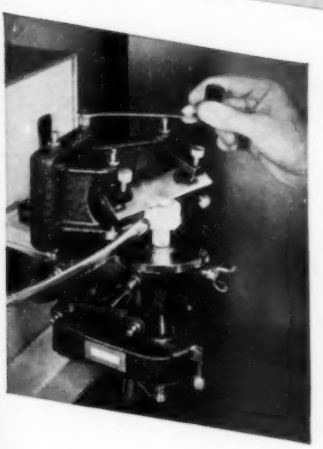
Quiet, compact unit for tissue freezing–

S/P Histo-Freeze





New, portable freezing unit for greater convenience in the pathology laboratory. Histo-Freeze maintains uniform rate of cooling. Compact unit occupies only 12″ x 18″ area; no noisy or cumbersome CO_2 tanks needed.

Fits all popular makes of microtomes; simple to install—microtome is easily secured to plate on front of Histo-Freeze for safe, vibrationless operation. Circulation of Freon 12 through unit holds temperature at approximately −30°F. Just turn compressor standby switch on in the morning and unit is ready for use any time during the day. In standby operation power consumption is just 50 watts. Lightweight—unit weighs only 50 lbs.

No. 67027A—Histo-Freeze, table model tissue freezing unit, complete, for use with AO freezing microtome. 115 volts, 60 cycles AC.	Each $450.00
No. 67027B—Same but for Bausch & Lomb microtome.	
No. 67027C—Same but for Sartorius microtome.	

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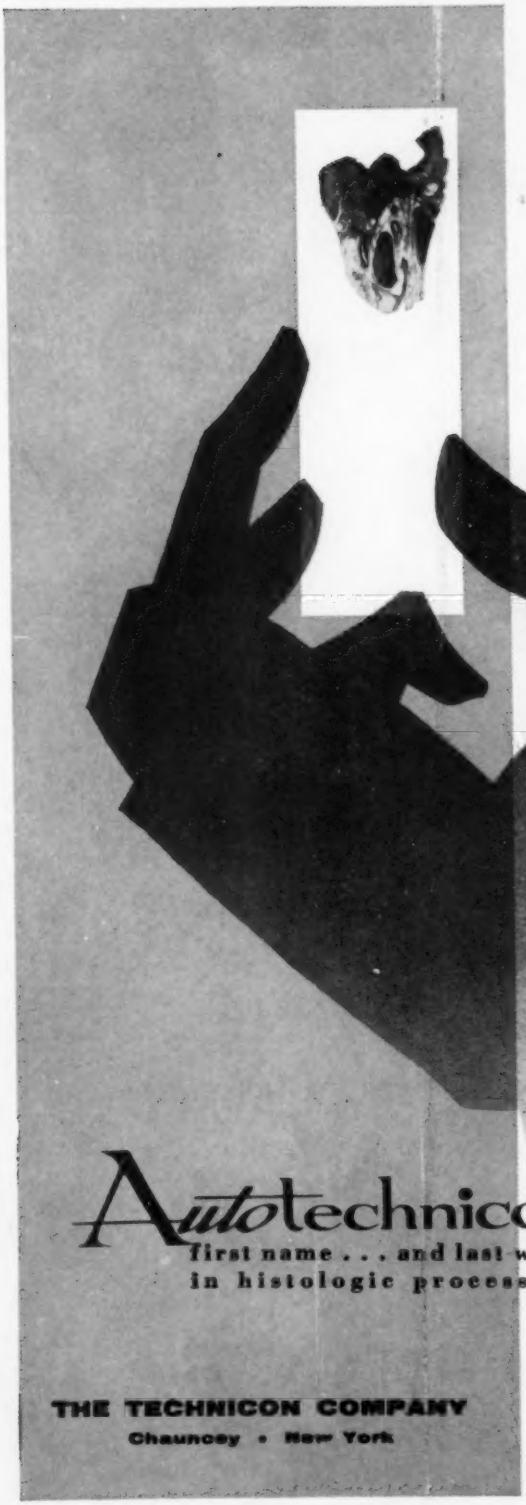

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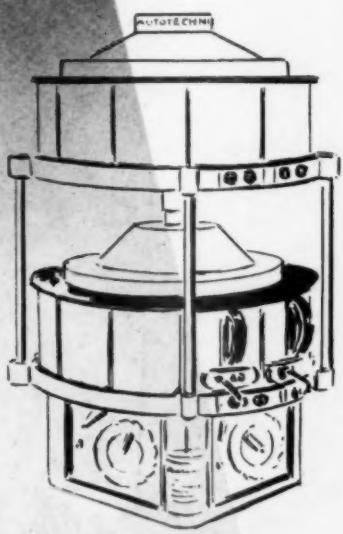

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